626

626
3-50.

Volume XIX SECTION B

303

JOURNAL

OF

THE MADRAS UNIVERSITY

ORIGINAL CONTRIBUTIONS IN MATHEMATICS, PHYSICAL AND BIOLOGICAL SCIENCES.

OFFICE OF THE REGISTRAR
12 AUG 1950
MADRAS

1383

DOCTRINA VIM PROMOVET INSITAM

PUBLISHED BY THE UNIVERSITY
MADRAS
1950

NOTICE

TO CONTRIBUTORS AND OTHERS

Citation shall be : *Jour. Madras Univ., Sect.* B, Vol.

Contributions, remittances, books for review, exchanges, and correspondence regarding all matters may be addressed to:

UNIVERSITY BUILDINGS
MADRAS

DR. C. P. GNANAMUTHU
Editor

Volume XIX SECTION B

JOURNAL

OF

THE MADRAS UNIVERSITY

Editor

DR. C. P. GNANAMUTHU, M.A., D.Sc., F.Z.S.

Editorial Board

DR. R. VAIDYANATHASWAMI, M.A., PH.D., D.Sc.

DR. T. S. SADASIVAN, M.Sc., PH.D.

DR. P. S. SARMA, M.Sc., PH.D.

DR. M. A. GOVINDA RAO, M.A., PH.D.

PUBLISHED BY THE UNIVERSITY

MADRAS

1950

JL
N50.19B.
93187

OFFICE OF THE REG... 12 AUG 1950 MADRAS

TABLE OF CONTENTS

I. SCIENCE

SOIL CONDITIONS AND ROOT DISEASES
By Miss T. S. Sarojini, B.Sc. (Hons.), Ph.D. .. 1

LARVAL STAGES OF SOME COPEPODS IN THE MADRAS PLANKTON AND THEIR SEASONAL FLUCTUATIONS
By S. Krishnaswamy, B.Sc. .. 32

FOOD FACTORS AND MIGRATION OF A FEW FISHES OF THE MADRAS COAST
By P. Vijiaraghavan, B.Sc. .. 59

MODULAR MULTIPLICATIVE FUNCTIONS
By C. S. Venkataraman, M.A., M.Sc. .. 69

SEED-BORNE FUNGI AND LOSS OF COTTON SEED VIABILITY
By C. S. Venkatram, B.Sc. (Hons.), M.Sc. .. 79

THE DIGESTABILITY AND AMINO ACID MAKE-UP OF CHEMICALLY MODIFIED KERATINS
By A. Narayanaswami, Ph.D. .. 113

II. TECHNOLOGY

SYNTHETIC DETERGENTS AND DETERGENCY
By P. B. Janardhanam, B.Sc. (Hons.) .. 132

SOIL CONDITIONS AND ROOT DISEASES

30

I. Micro-nutrient elements and disease development by *Fusarium udum* on Red gram [*Cajanus cajan* (Linn.) Millsp.].*

BY

(MISS) T. S. SAROJINI, B.Sc. (HONS.), PH.D.

(University Botany Laboratory, Madras)

Certain facts and hypotheses have emerged from the mass of recent experiments on all phases of plant and animal nutrition. Perhaps, the outstanding development of the last few years in agricultural science has been the recognition that many elements to which little attention was given in the past are quite as essential for the proper growth of plants as the 'big ten' of earlier times — carbon, hydrogen, oxygen, nitrogen, potassium, phosphorus, sulphur, magnesium, iron and calcium.

These newer factors in agriculture have been called the "minor", "rare", or "trace" elements. Hoagland (1944) comments that all these terms seem to be inappropriate and he prefers a general term "micro-nutrient" element, fully aware that objection can be found for this term also.

It would not be surprising if finally all the 92 elements will be found to have some effect upon plants, as the outstanding results obtained with some of these micro-elements have given sufficient impetus to renew the investigations concerning the role of many of the elements hitherto considered unessential.

Due credit should be given to the French School of workers — Bertrand (1927) on manganese, Javillier (1908) on zinc and Agulhon (1910) on boron — who were mainly responsible for awakening the interest regarding the function of these minute traces of elements, resulting in widespread investigations which are now of such practical importance.

Mazé (1914, 1915, 1919), McHargue (1919-1926), Warington (1923, 1926), Brenchley (1914), Brenchley and Warington (1927), Sommer and Lipman (1926) have furnished very convincing data to prove that these so-called unessential elements are essential to the growth and development of green plants.

Further, many workers have demonstrated that varying the nutrition of the plant has a decided effect on disease development.

* This paper formed Part of thesis in fulfilment of the Doctor of Philosophy Degree, University of Madras.

Wingard (1941) has reviewed comprehensively the literature on general disease resistance in plants. Recently Shear and Wingard (1944) have called attention to experimental evidence that brings out possible relationships between certain unbalanced conditions in the mineral nutrition of a host plant and the severity of attack by certain vascular pathogens. Noteworthy studies on this aspect are by Smith and Walker (1941), Young (1938) and Pryor (1940), who have convincingly proved that application of artificial fertilizers in the form of nitrate, phosphate or potash, is undoubtedly an effective way of reducing the proportion of crop lost through the agency of certain root diseases. The increase in plant resistance on supply of nutrients to the soil is attributed by Garrett (1941) mainly to stimulating a rapid production of fresh crown roots in place of those destroyed by disease, with the inclusion of an additional direct effect upon the resistance of individual crown roots to infection.

The majority of these investigations has been confined mainly to a study of the major elements ; comparatively very little work has been done with regard to micro-element and its nutritional bearing on host-parasite relationship. Millikan (1938) has reported considerable improvement on application of zinc sulphate in the growth of wheat crops suffering from the combined attack of a number of root-infecting fungi.

In a problem of this type where an intimate relationship exists between host and pathogen, independent studies should be made of (1) the fungus response to a substratum containing micro-elements (2) the reaction of the plant to the micro-element and (3) the response of the plant in the combined presence of the pathogen and micro-element.

Yogeswari (1948) working on the trace element nutrition of the wilt fungi — *F. vasinfectum, F. moniliforme* and *F. udum* from the pure culture aspect, demonstrated that all the three fungi responded well to various concentrations of zinc, boron, and manganese. Though optimum levels were low and differed somewhat with the three strains, yet higher concentrations were not toxic. It is, therefore, but natural to expect that further work along these lines would have a great bearing on the general understanding of host-parasite relationships in plant wilts.

It is commonly accepted that the essential elements are required only to supply the needs of plants for growth. It has been seen, however, that even an essential element becomes toxic, when supplied in high concentration. Also a physiological balance

of elements is very important in the choice of materials used as fertilizers.

To determine the indispensability or otherwise of a particular substance, especially in pure culture studies, it is very essential to determine the purity of the materials used. While in culture solutions, a more effective check could be exercised on the sources of introduction of these impurities, in glasshouse and field trials, the soil factor makes it impossible to exclude the presence of the elements even under highly controlled conditions.

In spite of this disadvantage, it was considered worthwhile to carry out the investigation as the same conditions would be met with in the field trials also.

The effect of boron, manganese and zinc on plant development and disease incidence by *F. udum* on red gram was therefore studied and the results are presented in this paper.

MATERIALS AND METHODS

The general laboratory technique followed was as proposed by Rawlins (1933), for preparation of media, inoculation, maintenance of cultures, etc. Some of the special methods used for the studies are detailed below. However, it may be mentioned that any technique over and above those given below will be referred to under experimental results.

I. *Micro-element nutrition and disease development in* Cajanus cajan :

Soil used : Coimbatore wilt-infested field soil collected in two seasons (November '46 and March '47).

Soil Containers : Earthenware pots (6·5″ × 7″ × 3·5″).

Moisture level : 75% moisture holding capacity of the soil.

Addition of micro-elements : Different concentrations of the three micro-elements tried, viz., boron, manganese and zinc in the form of boric acid, manganese sulphate and zinc sulphate, were obtained by dilutions with distilled water to give the respective parts per million levels. To each pot was added the required solutions of the particular strength to make up to 75% of the moisture content of the soil. The control series received distilled water.

The nutrient solutions were added initially and thereafter, subsequent loss in moisture was made good with necessary additions of distilled water every alternate day. The percentage of

pre-emergence wilt was calculated a fortnight after setting up the experiment.

The vigour of the plants affected by the micro-nutrient treatments was determined by taking the dry weight of shoots, one month after sowing of seeds.

II. *Micro-element nutrition and plant development*:

(1) *Cajanus* plants were grown in ordinary 'compost' soil to which addition of micro-nutrient solutions (favourable p.p.m. levels) had been made. After two months, the plants were transferred to the field and observations regarding flowering, seed weight and germination capacity of the seeds were made.

(2) *Cajanus* seeds were soaked in shallow dishes containing concentrations of micro-element solutions to observe if germination was hastened or delayed in any of the treatments. The seeds were soaked for different hours in the nutrient solutions and then sown in pots containing 'compost soil'. Weekly additions of the respective solutions were given to the pots and after 30 days, fresh and dry weights of the shoots were calculated to determine the growth of the plants.

III. *Micro-elements affecting survival and colonization of Fusaria on red gram stubble*:

The method for the investigation of survival and colonization of *F. udum* in soil was based on the technique devised by Sadasivan (1939) and later on used by Walker (1941) for *Fusarium culmorum*. For the survival tests infected red gram stem pieces were buried in soil to which was added micro-nutrient solutions. For colonization experiments, surface sterilized *Cajanus* stem bits were buried in soil watered with nutrient solutions. The moisture holding capacity in both cases was adjusted to 50% and only initial additions of nutrient solutions were made. The buried stubble was taken out at regular intervals and after surface sterilization, plated out to observe the extent of survival or colonization of the fungus under study.

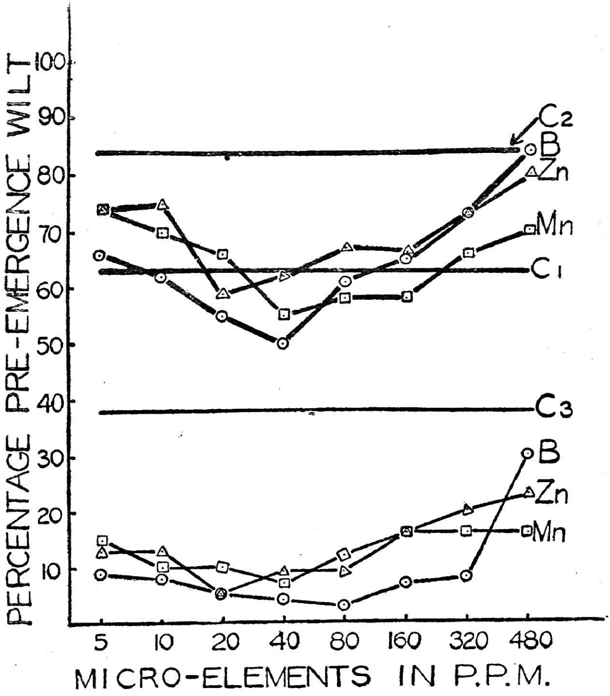
Experiment

I. *Micro-element nutrition and disease development in* Cajanus Cajan:

Experiment I: Soil collected in November 1946 from an infected red gram field in Coimbatore was used in this experiment. To ensure infection, additional inoculum was supplied. The pots were run in triplicate and 25 pre-soaked seeds were sown in each pot, twenty-four hours after inoculation and addition of nutrient

solutions. Two controls were maintained — (1) seeds sown in plain wilt soil and (2) seeds sown in inoculated wilt soil. (Inoculum was obtained by infecting red gram stem pieces soaked in 1% sugar solution with *F. udum* in pure culture and incubating them for 3 weeks at 29-30°C. 5% inoculum by weight of soil was added to each pot.)

The above experiment was repeated with soil collection obtained from the same field in March 1947. Additional inoculum was found to be unnecessary as the soil itself was capable of causing considerable pre-emergence wilt. The results are presented in Text — fig. I.



Text — fig. I: Percentage pre-emergence wilt of *Cajanus* in wilt-sick-soil (November 1946 and March 1947) with regard to addition of micronutrient solutions of boron, manganese and zinc at various levels.

The micro-nutrient treated series on the whole yielded better results than the control, by lowering the disease index to an appreciable extent and at the same time promoting plant growth.

Four important observations can be made from Text fig. I.

(a) In all the three micro-nutrient treatments a distinct improvement was seen in percentage emergence over the control series.

(b) A decrease in pre-emergence wilt of the inoculated micro-nutrient treatment over the control [wilt soil] was seen between 20 and 80 p.p.m. levels in all the three cases.

(c) Boron though showing the lowest figures for pre-emergence wilt yet produced toxic symptoms like marginal scorching of leaves at the 40 p.p.m. level itself, the lower leaves being affected first. The toxicity increased with highest dosage used in the series.

(d) Manganese treatment was seen to be the most beneficial followed by zinc and boron in the decreasing order of efficiency. The elements, manganese and zinc did not produce any apparent toxic symptoms even with the highest dosage used in the series.

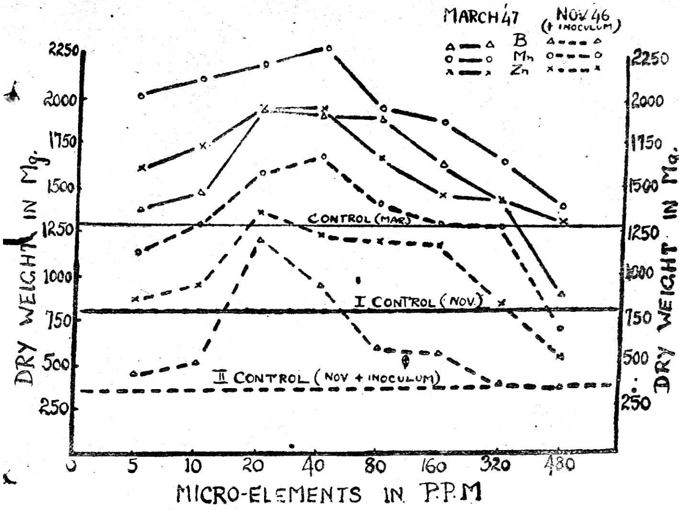
Eaton (1940) also observed that symptoms for boron toxicity appeared first on the mature leaves at the base of the plant and he stated that the greatest growth may be at concentrations that were clearly toxic as shown by the effects on the leaves.

The greater protection observed in the lower doses of the micro-nutrients viz., 20, 40 and 80 p.p.m. was considered to be due to increased plant growth (vigour) in the presence of a more complete nutrition. It was, therefore, necessary to ascertain if plant vigour improved when supplied with micro-elements. This was determined by estimating the dry weight of shoots 30 days following sowing of seeds. For dry weight estimations of *Cajanus* plant material made in this study, shoot lengths were obtained by cutting one inch below the first-leaf node. The material was then dried in an electric oven at 135°C., for 2 hours (Anon, 1935).

Text — fig. II shows the dry weight of shoots belonging to the November and March trials respectively (mean of 3 replicates, 5 plants for each replicate).

Results: An appreciable increase in dry weight was recorded in the treated series, especially at the lower levels of the three elements.

Optimum dry weights were recorded at 20 p.p.m. for boron and zinc and at 40 p.p.m. for manganese



Text — figure II: Dry weight estimation of *Cajanus* shoots belonging to the November 1946 and March 1947 trials.

There is a wide variation seen in percentage emergence as well as the dry weight estimations between November and March trials. It may be argued that the former was given additional inoculum and hence the lower figures. But the controls of the respective series also show this difference. The improvement seen with the March soil collection can be due possibly to the varying climatic and soil conditions prevalent in the two periods of soil collection. The fallow season in Coimbatore for the *Cajanus* crop is from the month of February to May. During these dry summer months, the fungus must necessarily exist saprophytically as soil-borne mycelium. In this state, its persistence depends upon the presence of favourable soil conditions such as sufficient moisture, to prevent death from dessication.

Elmer (1942) has correlated the saprophytic persistence of *Rhizoctonia solani* in the soil with summer weather conditions. He states that a high percentage of infection in any year could be correlated with the occurrence of soil conditions suitable for saprophytic survival of *R. solani* mycelium in the soil during July and August in the preceding year, viz., high rainfall, low temperatures, and absence of drying winds.

March conditions in Coimbatore are far from ideal for the fungus. On the other hand, in November, when an almost mature crop is on the field, the soil and environmental conditions are very satisfactory. Low temperatures prevail with good showers. The parasitic activity of the pathogen is then at its greatest, as shown by the high percentage of wilt occurring in that season. The crop is harvested in early January. As the drought sets in, fungal activity declines, the general micro-biological activity in the soil contributing to the rapidity of its disappearance and loss of viability.

Nevertheless, it is interesting to note that irrespective of the degree of infectivity of the soil, a definite improvement can be obtained in the micro-nutrient series over the control.

Experiment II: The response of the plants (growing in soil collected in March) to combinations of the micro-elements, (boron + manganese + zinc) at the favourable levels — 20, 40 and 80 p.p.m. — was then studied. The nutrient solutions of the three elements were combined on a quantitative basis to make up the respective levels collectively.

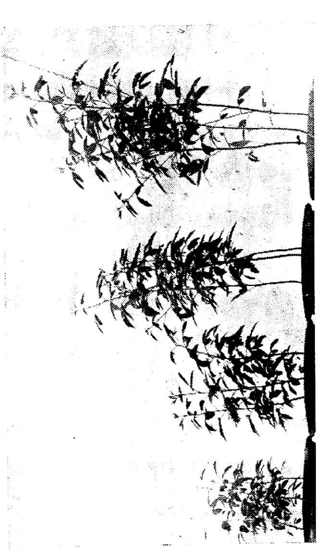
The percentage emergence and plant vigour were determined a month following sowing of seeds. The results are presented in Text — fig. III.

Results: The figures obtained for emergence were higher than the control in all treatments. The percentage emergence in the 20 p.p.m. level was lower than that obtained in the individual micro-nutrient treated experiment with the same soil collection. At 40 p.p.m., the emergence was 100%, while at the 80 p.p.m. level, it fell to 92%. Slight boron-toxicity symptoms were observed in the two higher levels — 40 and 80 p.p.m.

The dry weight estimates of seedlings were almost equal to those obtained in the "individual" treatments. Here also, the 40 p.p.m. level (B + Mn + Zn) yielded highest figures for dry weights.

On the whole it was observed that treatment with the individual micro-element was more favourable for seed emergence and plant development in infected soil, than with the "combination".

This may probably be due to the fact that the pathogen was benefitted more by additions of nutrient solutions of the elements in combination than when added individually. Indeed, Yogeswari (1948) has observed increased mat production of *F. vasinfectum*, *F. moniliforme* and *F. udum* in pure culture, with additions of



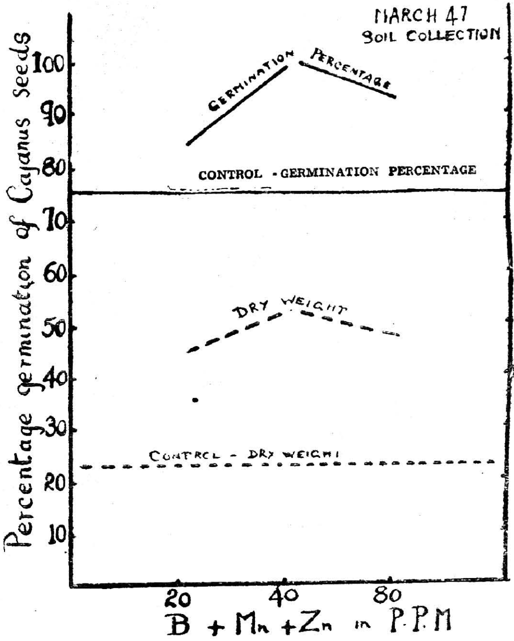
Control 80 p.p.m. 40 p.p.m. 20 p.p.m.

Fig. 2

Control 80 p.p.m. 40 p.p.m. 20 p.p.m.

Fig. 4

boron, manganese and zinc in combination than when supplied singly.



Text — figure III: Percentage emergence of *Cajanus* in wilt-sick-soil (March 1947) and dry weight estimation of shoots in relation to "combination" micro-element treatment (B + Mn + Zn)

II. *Micro-element nutrition and plant development.*

Experiment I: 1. *Pot trial.* The response of the plants alone with regard to micro-nutrient treatments was studied by adding the nutrient solutions of the three elements in combination and separately at levels of 20, 40 and 80 p.p.m.

The heights of plants were taken after 60 days growth in pots before they were transferred to the field.

Plate I figs. 1 — 4 show the growth of the plants in the various treatments at the end of 2 months.

Table 1 giving the height of red gram plants treated with 20, 40 and 80 p.p.m. levels of B, Mn and Zn, separately and in combination (mean of 5 replicates — 3 plants for each replicate).

Micro-Element	Levels in p.p.m.	Height
	20	2′
Boron	40	2′4″
	80	1′5″
	20	3′
Manganese	40	2′5″
	80	1′9″
	20	2′5″
Zinc	40	2′3″
	80	2′1″
	20	3′
B + Mn + Zn Combination	40	3′5″
	80	2′
Control	..	1′4″

Results: (a) Manganese seemed to be most beneficial of all the treatments at the 20 p.p.m. level, while at the 40 p.p.m. level, the combination treatment produced the tallest plants of the entire experiment.

(b) In the boron series, plants were tallest at the 40 p.p.m. dosage, but boron injury was seen in the lower leaves.

(c) In the 80 p.p.m. level, manganese and combination treatments (B + Mn + Zn) gave almost identical results while the figure for zinc was slightly higher. This dosage was definitely harmful in the case of boron, the plants becoming stunted with small leaves crowding together and on the whole manifesting typical symptoms of boron toxicity (Plate II, fig. 5).

The combination treatments at the 40 and 80 p.p.m. levels, in spite of producing the tallest plants of the entire series, exhibited some boron injury in the form of marginal scorching of the lower leaves. Compared with the individual boron treatment, the injury was very slight, probably due to the presence of the other micronutrients. (Plate II, fig. 7).

Fig. 5

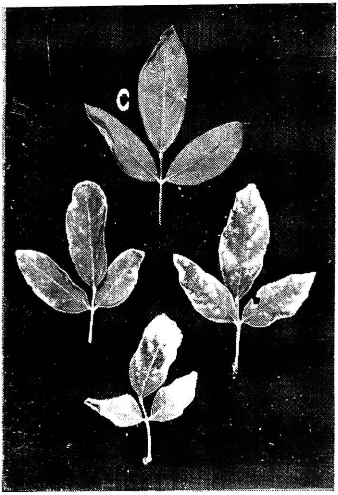


Fig. 6

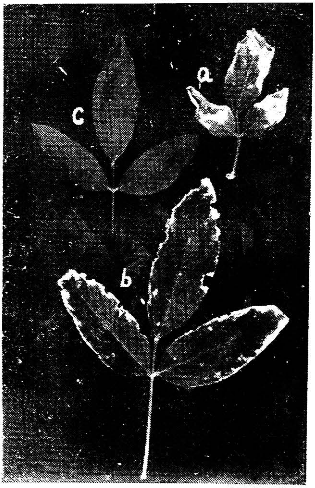


Fig. 7

T. S. SAROJINI

PLATE II

2. *Plot Trial*: Observations were made to determine whether the initial micro-element nutrition affected the normal development of the plant after being transferred to the field. The period of flowering was noted in all the cases and is presented in Table 2.

Table 2 showing the time taken to flower by *Cajanus* plants treated with micro-nutrient solutions of B, Mn, and Zn, separately and in combination (variations observed in 15 plants per treatment).

Micro-Element	Levels in p.p.m.	Days after sowing
Boron	20	143 — 147
	40	146 — 155
	80	149 — 178
Manganese	20	144 — 145
	40	138 — 144
	80	138 — 144
Zinc	20	148 — 150
	40	146 — 151
	80	141 — 147
B + Mn + Zn	20	130 — 132
	40	120 — 123
	80	135 — 138
Control	..	149 — 155

Results: The plants belonging to the 40 p.p.m. combination treatment were earliest to flower whereas, plants treated with manganese alone, followed closely after. There was not much difference in the zinc and boron series when compared with the control, except that in the highest level of boron treatment, the time taken to flower by most of the plants was prolonged. Moreover, these plants were very stunted and put forth flowers on only few of the branches. Consequently seed output was markedly less in this treatment.

3. *Plant nutrition and seed production*:

Seeds were collected from plants belonging to the various treatments for determination of their fresh and dry weight. Prior

to estimation of seed weight, they were dried in the sun for two days (4 hours daily). 500 seeds in 5 lots of 100 each were weighed for every treatment and the fresh and dry weight figures obtained are presented in Table 3.

Table 3 giving fresh and dry weights of seeds collected from treated plants.

Micro-Element	Levels in p.p.m.	Fresh weight in g.	Dry weight in g.
	20	7·192	6·526
Boron	40	7·392	6·772
	80	7·688	6·36
	20	7·722	6·923
Manganese	40	7·7	7·054
	80	7·964	7·109
	20	7·347	6·691
Zinc	40	7·425	6·819
	80	7·251	6·674
	20	7·325	6·867
B + Mn + Zn	40	7·357	6·876
	80	7·634	7·262
Control	..	7·275	6·564

Results: The fresh and dry weight of seeds collected from the treated plants were on the whole higher than that obtained for the control.

Altogether highest figures were obtained in the manganese treatment. In the combination treatment, seeds belonging to the 80 p.p.m. level treated plants compared favourably with those obtained for the manganese series. The dry weight value obtained at this level was the best for the whole experiment. Zinc and boron behaved almost similarly as regards fresh weight figures which were not very much above that of the control. On dry weight basis, however, zinc treatment gave better results than boron.

Boron did not seem to be beneficial as the values obtained for dry weights were lower than the control at two of the three levels tried — viz., at 20 and 80 p.p.m.

4. *Plant nutrition and germination capacity of seeds*:

The germination capacity of seeds collected from micro-nutrient treated plants was tested. 500 seeds in 5 lots of 100 were sown for each treatment in pots containing 'compost' soil. Percentage germination was observed upto two weeks after sowing.

Table 4 showing the percentage germination figures obtained for seeds collected from plants treated with micro-nutrients.

Micro-Element	20 p.p.m.	40 p.p.m.	80 p.p.m.
Boron	80	76	65
Manganese	80	90	92
Zinc	90	92	92
B + Mn + Zn	80	88	86
Control........................80			

Results: The germination capacity of seeds collected from micro-element treated plants was on the whole better than the control in all treatments except the two higher levels of boron, where comparatively lower figures were obtained. The individual micro-nutrient treatments yielded better results than the "combination" series regarding germination capacity of red gram seeds.

Experiment II: 1. *Micro-element and germination of Cajanus seeds*: To determine whether trace elements had any effect on germination, *Cajanus* seeds were soaked in various strengths of the nutrient solutions for 24 hours, after which counts were made of the number germinated. The levels tried were 0·5, 1, 2, 5, 20, 40, 80, 160, 320 and 480 p.p.m. Controls were maintained with distilled water. (For combination treatment, only 20, 40 and 80 p.p.m. levels were tried.)

Table 5 showing the germination percentage of red gram seeds after 24 hours soaking in various levels of micro-nutrient solutions (mean of four replicates — 25 seeds for each replicate).

Micro-Element	PERCENTAGE GERMINATION OF SEEDS									
	Levels in p.p.m.									
	0·5	1	2	5	20	40	80	160	320	480
Boron	40	42	45	56	50	32	28	26	8	6
Manganese	40	48	56	63	60	60	46	33	27	22
Zinc	42	46	48	54	60	64	56	44	40	32
B + Mn + Zn					46	46	44			
Control......30										

Results: The germination figures obtained for seeds soaked in plain distilled water were lower than for seeds supplied with micro-nutrient solutions at most of the levels tried.

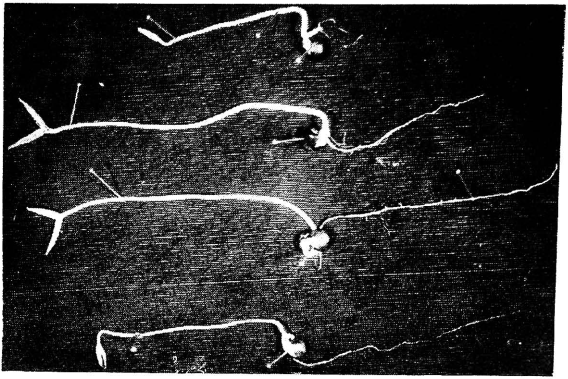
Seeds soaked in manganese and zinc nutrient solutions exhibited best germination capacity especially between 2 and 80 p.p.m. levels. The optimum for manganese and boron was at 5 p.p.m while it was at 40 p.p.m. for zinc.

Boron was beneficial up to 20 p.p.m. after which it produced an inhibitory effect, the germination figures at 80 p.p.m. and higher levels being even lower than those of the control and extremely poor at the 320 and 480 p.p.m. levels.

Lower figures than the control at these two levels were obtained with manganese also.

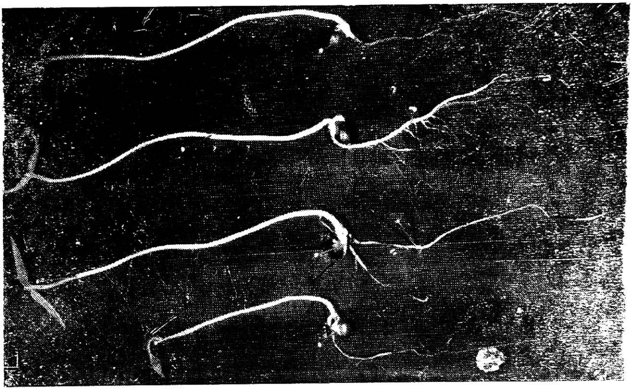
The three levels of the combination treatment were not harmful but the improvement in germination noted over the control when the elements were supplied singly was not obtained here to the same extent.

The nutrient solutions not only affected germination to some extent but also the subsequent vigour of the seedling. Plate III, figs. 8 — 10 show the development of seedlings from seeds soaked in various concentrations of the micro-element boron. Upto 5 p.p.m., the nutrient solution was very beneficial, after which seedling development was affected, becoming more harmful as the levels increased in concentration. Although a somewhat depressing influence was produced by zinc and manganese at the very high levels, yet it was not so marked as in the case of boron. The combination levels as seen previously were not injurious and produced better developed seedlings than the control.



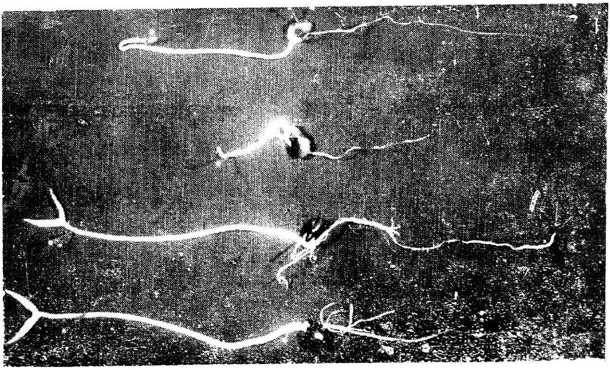
g h i Control

Fig. 10



Control d e f

Fig. 9



Control c b a

Fig. 8

T. S. SAROJINI

PLATE III

Micheels and Heen (1906) found that not only a greater percentage of germination was obtained with addition of manganese but there was also a decided increase in the weight of the seedlings and the length of root development. They ascribed the stimulating action of manganese to a diastatic role acting upon the reserve material of the seed.

2. *Duration of soaking in nutrient solutions affecting seed germination and vigour of seedling* :

The effect of varying the hours of soaking in the different micro-nutrient solutions on germination capacity of the seeds and resultant vigour of seedling was then studied.

Cajanus seeds were soaked in nutrient solutions of the elements boron, manganese and zinc at the favourable concentrations — 2, 5, and 20 p.p.m. — for 8, 16 and 24 hours after which they were simultaneously sown in pots containing ordinary 'compost' soil and incubated at 20-30° C. Moisture level was maintained at 50%. There were four replicates for each treatment and 10 seeds were sown per pot. The results are presented in Table 6.

Table 6 showing the germination of seeds soaked for 8, 16 and 24 hours in the micro-nutrient solutions.

Micro-Element	Levels in p.p.m.	PERCENTAGE GERMINATION OF SEEDS		
		8 hours	16 hours	24 hours
	2	85	78	78
Boron	5	84	81	81
	20	92	81	78
	2	78	78	83
Manganese	5	78	79	80
	20	78	78	81
	2	74	89	74
Zinc	5	85	81	78
	20	78	89	81
Control — soaked		79	74	74
Control — unsoaked.........74				

Results : In the boron treatment, with longer hours of soaking in all the levels there was a fall in germination, while the reverse was seen in the case of manganese. In zinc, however, the first two periods were more favourable than a 24-hour soaking.

Definite improvement over the control was observed better in the 16 and 24 hours soaking trials than in the 8-hour series where, except for the boron treatment in all concentrations and zinc at 5 p.p.m. level, all the figures obtained were slightly lower than the control. In the control series soaking for 8 hours gave better results for germination when compared with the unsoaked trials.

The fresh and dry weights of shoots were estimated a month following sowing of seeds to determine the vigour of seedlings as affected by pre-soaking of seeds in various concentrations of micro-nutrient solutions for different hours.

Table 7 showing the fresh and dry weights of shoots obtained from seedlings developing from seeds pre-soaked in 2, 5 and 20 p.p.m. levels of B, Mn, and Zn for 8, 16 and 24 hours.

Micro-Element	Levels in p.p.m.	8 hours		16 hours		24 hours	
		Fresh weight in g.	Dry weight in g.	Fresh weight in g.	Dry weight in g.	Fresh weight in g.	Dry weight in g.
	2	1·4	0·43	2·1	0·5	2	0·42
Boron	5	1·65	0·5	1·7	0·4	1·6	0·3
	20	1·12	0·42	1·6	0·3	1·3	0·3
	2	1·72	0·52	1·8	0·4	2·05	0·55
Manganese	5	2·08	0·7	2·05	0·6	2·06	0·6
	20	2·32	0·6	1·8	0·4	1·8	0·4
	2	1·6	0·5	2·1	0·5	1·8	0·3
Zinc	5	1·75	0·55	1·8	0·4	1·8	0·3
	20	2·3	0·6	2	0·5	1·8	0·45
Control — soaked		1·6	0·4	1·6	0·5	1·68	0·4
Control — unsoaked		1·4	0·4				

Results : Manganese on the whole, was seen to be the most beneficial of all the three elements for both seed germination and development.

Zinc was almost as good as manganese and was definitely better than boron.

Boron at the lowest level was not injurious even with prolonging the soaking period. But at 5 and 20 p.p.m., there was a fall especially in dry weight figures with increase in hours of soaking.

Manganese yielded better results with extension of the soaking period except in the highest concentration, where the 8-hour soaking produced higher shoot weights than 16 and 24 hours. The 5 p.p.m. level was favourable on the whole.

Zinc treatment was beneficial up to 16 hours soaking, while after that, a fall in shoot weight was recorded. Optimum level for zinc was between 5-20 p.p.m.

The fresh and dry weights of shoots of "soaked" as compared with those obtained for "unsoaked" control did not differ much, although a slight increase was observed on fresh weight basis.

The results presented have shown that the elements under study are indispensable for normal development of the red gram plant to minimise the attack of the causal pathogen, *F. udum,* to a considerable degree. Of the three elements tried, manganese was seen to be the most beneficial. Zinc also yielded satisfactory results, though not to the same extent as manganese. Boron, however, was very injurious if supplied above 20 p.p.m.

Manganese has been claimed by several workers as the most important and necesssary factor in the synthesis of chlorophyll and carbon assimilation. McHargue (1926) found that none of the common elements, including iron, copper, zinc boron or arsenic could replace this function of manganese in the growth of plants. He conclusively proved that manganese played a role of importance equal to that of iron in the synthesis of chlorophyll and that it occurred in the largest concentrations in the leaves and seed-germs of plants. He also observed that leguminous plants were more sensitive to the lack of manganese, thus suggesting that the element was apparently concerned in N_2 assimilation and synthesis of proteins.

A marked beneficial response was obtained by Brenchley (1914), Sommer and Lipman (1926), Arnon (1938) and Haas (1937) with the application of zinc. Zinc was found not only to stimulate plant development but also to influence the activities of soil microflora as well — (Chandler, 1937; Millikan, 1938, 1942).

The element boron has had comparatively more attention than manganese and zinc in agricultural science. Several investigators studied the influence of boron, particularly on leguminous crops and arrived at the conclusion that although traces of boron were necessary, yet, it would become toxic if present in slightly larger doses. Leguminous plants were found to be more sensitive to boron than any other crop plant (Cook 1915; Dennis, 1935-37). That boron was toxic to other crop plants as well at slightly higher

levels was observed by Wolfe (1940) for cauliiflower and radish, Conner (1917) for corn and Brown (1922) for potatoes. Applications of borax (60 lb. per acre) was found by Heggeness (1942) to prevent development of rust on flax. This concentration resulted in some burning of the leaf tips but it was felt that the concentration of the element could be reduced without losing the protective effect.

The results obtained in the course of this study of the indispensability of these trace elements in the light of plant nutrition and disease development in *Cajanus cajan*, though not of great value until after confirmation with large scale field trials, nevertheless, are of interest in indicating a new line of attack of the wilt problem.

III. *Micro-elements affecting survival and colonisation of Fusaria on red gram stubble.*

A real understanding of the true relation of soil conditions to disease can only follow a study of the pathogen under conditions generally obtaining in natural soils which give full scope for the interaction of the various chemical, physical and biotic factors of the soil. Many investigators have studied the parasitic activity of the root-infecting fungi; their saprophytic mode of persistence in soils under natural conditions has received but scant attention. Garrett (1944) has pointed out that two types of saprophytism can be distinguished amongst the root-infecting fungi :—

(1) active saproyhytic colonisation of dead plant tissues lying on or in the soil, and

(2) limited saprophytic survival in tissues originally invaded as parasite.

The former type of behaviour is typical of the more primitive parasites of the *soil inhabitant* class and the latter of the more specialized parasites of the *soil invader* class.

Rosellinia sp., *Rhizoctonia solani* and *Fusarium culmorum* may be selected as furnishing the best known examples of the three types of active saprophytism where they make a macroscopically or microscopically visible or no extensive spread at all through the soil respectively (Nowell, 1923 ; Blair, 1943 ; Sadasivan, 1939). In the case of fungi which do not make any appreciable growth in soils, for instance the Fusaria, the question arises as to how they can survive effectively in soils. Their chances of survival seem to be limited mainly to their persistence as vegetative mycelium, conidia, chlamydospores and sclerotia or as a saprophyte on organic plant residues in soil. Subramanian (1946 a) investigating the fate of *F. vasinfectum* when introduced in natural unsterilized soil,

using the Cholodny slide technique, found that the hyphae, conidia and chlamydospores were decomposed sooner or later, by the antagonism of the soil microflora, particularly the bacteria. In general, the disappearance of mycelium or spores of *F. vasinfectum* in the soil was proportional to the general soil microbiological activity. Although its persistence was limited by the operation of the microbial factor, yet it was found that it could colonize a variety of plant material under a wide range of soil conditions. Due to its prolonged persistence as a saprophyte, Subramanian (1946 b) found it justifiable to include it in the class of true soil fungi or *soil inhabitants.*

How then would *Fusarium udum* react under identical conditions was the question to be settled ? The active saprophytic colonization of the pathogen on host stubble was taken up for investigation. Subramanian (1950) and Zachariah (unpublished) had already observed that 100% colonization of *Fusarium* could be obtained within one to two weeks of burial of cotton stubble in Udamalpet and Coimbatore cotton wilt sick soils. In pure culture the vegetative ability of *F. udum* was known to be catalysed by the addition of micro-elements like boron, zinc and manganese at very low concentrations (Yogeswari, 1948). Similar results were obtained by Sulochana (unpublished) on increased sporulation of *F. vasinfectum* in the presence of traces of micro-elements like aluminium, boron, manganese, zinc, arsenic, etc. Although increased mycelial activity (denoted by increased mat weight) and increased sporulation (denoted by increase in spore numbers) were seen at low concentrations, this beneficial effect went down as the concentration increased. It was, therefore, considered that a study on the behaviour of *F. udum* colonizing stubble, on the addition of micro-nutrient elements to infected soils would throw light on its ability for saprophytic activity. It must be mentioned here that this colonization and survival of wilt fungi in soils has been a vexed problem and no direct correlation has so far been obtained between their presence in soils and the factors that limit their colonization on residual plant material.

Experiment I : Surface sterilized red gram stem pieces were buried in pots containing infected soil (March '47 collection). The 20, 40 and 80 p.p.m. levels found to be favourable for pre-emergence wilt control in the previous experiment were chosen for investigation. Initial additions of micro-nutrient solutions of the 3 elements at the 3 levels were added and the moisture holding capacity was adjusted at 50%. Thereafter, the loss in moisture was made good with necessary additions of distilled water. 25 gram stem pieces were buried per pot and plated out at monthly intervals.

Table 8 showing the colonization of *Fusarium* on red gram stem pieces buried in infected soil as affected by the addition of micro-nutrient solutions (boron, manganese and zinc at 20, 40 and 80 p.p.m. levels).

Micro-Element	Levels in p.p.m.	Incubation Period in Months			
		1	2	3	4
	20	20	8	8	34
Boron	40	16	25	66	84
	80	4	20	44	76
	20	26	35	77	92
Manganese	40	4	20	76	88
	80	16	37	84	92
	20	20	34	13	..
Zinc	40	12	8	..	..
	80	12	7	..	..
Control		100	100	100	100

Results: At the outset, the colonization of *Fusarium* in the micro-element treatments was very much lower than the control which continued to show 100% colonization throughout the duration of the experiment. With increase in level of the micro-element added, there was a fall in percentage colonization of *Fusarium.*

In boron and manganese treatments, the colonization percentage increased from the first month after burial and at the end of four months was nearly 100% in all the cases except the lowest level of boron.

Zinc, however, seemed to have an adverse effect on the colonization of *Fusarium,* as the percentage began to fall from the second month onwards. This was very marked in the two higher levels (40 and 80 p.p.m.) where there was no colonization by *Fusarium* in the third and fourth months, while at the 20 p.p.m. level this was observed only from the fourth month onwards.

In the boron and manganese treatments, other soil fungi like *Mucor* sp., *Rhizoctonia* sp. and *Cunninghamella* sp. appearing along with *Fusarium* on the stubble, gradually showed a decrease in percentage colonization, as *Fusarium* increased in appearance. The fungi that tended to increase with period of burial were *Aspergillus niger* and *Pencillium* sp. and this increase was seen in the boron and zinc treatments. In the manganese treatment however, where highest percentage colonization of *Fusarium* was obtained, all the other fungi that appeared after the first month exhibited considerable fall in the percentage colonization with increase in period of burial in soil. This was also seen in the control.

Table 9 giving the percentage colonization of other soil fungi in the various treatments is presented in the next page.

Micro-Element	Levels in p.p.m.	Details of colonization	Sampling period in months			
			1	2	3	4
Control		Mucor sp.	100	100	100	32
		Aspergillus niger	60	36	100	24
		Penicillium sp.	4	28	48	44
		Rhizoctonia sp.	..	..	..	..
		Cunninghamella sp.	12	8	..	..
Boron	20	Mucor sp.	100	76	100	24
		Aspergillus niger	36	24	68	56
		Penicillium sp.	12	48	92	84
		Rhizoctonia sp.	..	12	..	..
		Cunninghamella sp.	24	..	..	..
	40	Mucor sp.	100	32	..	64
		Aspergillus niger	8	16	13	24
		Penicillium sp.	48	40	52	80
		Rhizoctonia sp.	..	..	..	..
		Cunninghamella sp.	..	..	..	..
	80	Mucor sp.	100	60	72	36
		Aspergillus niger	4	40	40	76
		Penicillium sp.	24	20	64	80
		Rhizoctonia sp.	..	5	..	..
		Cunninghamella sp.	16	..	..	..
Manganese	20	Mucor sp.	100	84	100	76
		Aspergillus niger	..	64	..	24
		Penicillium sp.	..	56	56	12
		Rhizoctonia sp.	..	..	..	..
		Cunninghamella sp.	..	..	..	..
	40	Mucor sp.	100	100	48	48
		Aspergillus niger	72	24	48	24
		Penicillium sp.	36	72	32	12
		Rhizoctonia sp.	..	..	..	..
		Cunninghamella sp.	..	..	..	..
	80	Mucor sp.	100	84	100	76
		Aspergillus niger	92	32	28	8
		Penicillium sp.	68	12	80	28
		Rhizoctonia sp.	..	..	..	..
		Cunninghamella sp.	..	..	..	..
Zinc	20	Mucor sp.	100	41	12	12
		Aspergillus niger	44	80	84	80
		Penicillium sp.	64	28	68	84
		Rhizoctonia sp.	..	..	..	..
		Cunninghamella sp.	16	..	..	..
	40	Aspergillus niger	100	92	12	..
		Mucor sp.	72	96	100	100
		Penicillium sp.	68	64	64	60
		Rhizoctonia sp.	..	..	..	..
		Cunninghamella sp.	16	..	..	..
	80	Mucor sp.	100	95	24	..
		Aspergillus niger	60	70	100	100
		Penicillium sp.	36	36	32	36
		Rhizoctonia sp.	..	..	..	..
		Cunninghamella sp.	..	4	..	..

The distinct fall in *Fusarium* colonization obtained with addition of zinc nutrient solutions seemed to compare favourably with the results obtained by Millikan (1938, 1942) in Australia. As has been previously referred to, Millikan (1938) observed that on addition of zinc sulphate (15-20 lb. per acre), a definite improvement was noticed in wheat crops attacked by several root-infecting pathogens.

Experiment II : The survival of the pathogen in soil, in the presence of the minor elements was then studied. Infected host stubble was buried in garden "compost" soil in pots and supplied with nutrient solutions at the three levels — 20, 40 and 80 p.p.m. — to make up to 50% of the water holding capacity of the soil. Subsequent loss in moisture was made good with necessary additions of distilled water. Weekly platings (25 pieces per treatment) of the buried stubble were made up to two months to determine the survival period of *F. udum* under the above experimental conditions.

Table 10 showing percentage survival of *F. udum* on *Cajanus* stubble with addition of micro-nutrient solutions of boron, manganese and zinc at 20, 40 and 80 p.p.m. levels.

Micro-Element	Levels in p.p.m.	Period of incubation in weeks							
		1	2	3	4	5	6	7	8
	20	96	84	72	56	32	16	12	4
Boron	40	96	80	76	56	36	12	..	..
	80	90	80	76	62	32	8	..	..
	20	90	84	76	64	36	20	12	12
Manganese	40	92	84	76	64	40	24	16	4
	80	90	80	72	60	40	20	16	8
	20	75	57	38	12	4	..	..	..
Zinc	40	76	48	32	8	..	..	..	..
	80	75	47	30	6	..	..	..	..
Control		100	100	96	88	84	80	76	76

Results : In the micro-element treatment, the disappearance of the fungus was hastened to a considerable extent over the control.

There was no appreciable difference in the behaviour of the pathogen with variation in levels of the nutrient solution. Most rapid disappearance of *F. udum* was brought about with addition of zinc nutrient solutions, the fungus being exterminated within four to six weeks of burial in the soil.

It has, however, been demonstrated in this laboratory (Zachariah, unpublished) that *F. vasinfectum*-infected cotton stubble exhibits 100% survival even at the end of six months incubation in the soil under natural field conditions. Further, work done on similar lines to study the additional effect on micro-elements on the survival of *F. vasinfectum* in Udamalpet cotton wilt-sick soil (Sulochana, unpublished) has revealed that the survival of the cotton-wilt pathogen is unaffected upto three months burial in soil. The elements tried included boron, manganese and zinc at levels of 50 to 350 p.p.m. After four months, a fall in survival by 40 and 30% with zinc and boron treatments respectively, was noted. The other elements were found to be ineffective in lowering the survival capacity of the pathogen.

The survival of *Rhizoctonia bataticola* on cotton stubble buried in "compost" soil as affected by micro-element solutions, however, yielded similar results as those obtained by the author in connection with survival of *F. udum*. It was observed (Radha, unpublished) that almost total disappearance of the fungus could be obtained within eight weeks of its burial in soil, especially in the zinc and boron treatments.

The contradictory nature of the results obtained with *F. vasinfectum* and *F. udum* could be justified only by the explanation that survival could be affected by the nature of the soil in which the infected stubble was buried, the nature of the stubble used for colonising the fungus in pure culture and the more efficient survival capacity of one particular pathogen over the other.

That different soils may influence survival of the pathogen to some extent was reasonable to hold, but even here, the results obtained by Zachariah (unpublished) with survival in "compost" soil for *F. vasinfectum*, when compared with those obtained for *F. udum* under identical conditions, failed to substantiate this.

Discussion

In conclusion, it is necessary that the results obtained during the course of this study be viewed in the light of other investi-

gations, past and present, which would ultimately lead to a better understanding of such problems in the future. An attempt is made to discuss the more important observations in relation to pigeon-pea wilt control and wilt diseases in general.

Pigeon-pea is exclusively grown in India and to a lesser extent in the neighbouring countries to the East and South East. Where-ever the crop is grown, considerable wilt occurs resulting in highly reduced yields. Cotton, which also suffers from a somewhat similar disease, on the other hand, is grown all over the world and so has had more attention bestowed on it, particularly from the wilt control aspect. As for pigeon-pea wilt, the problem has been left untouched almost, but for the work done at Delhi (Anon, 1940).

This dearth of scientific investigation looks all the more striking when in almost all the provinces where *Cajanus* is cultivated, there is a deficit in yield of more than half the amount required for normal consumption. In the Madras Province, for instance, for a consumption of 1,22,500 tons of this pulse, the average production is 39,300 tons per annum, leaving thereby a deficit of 83,200 tons. Comparison of these figures with production in foreign countries is not possible (like in cotton) as cultivation of pigeon-pea is mostly restricted to India. The yield per acre is no doubt dependent on various factors like soil fertility etc.; but the fact that wilt also considerably reduces the stand and thereby yield of crops, cannot be overlooked.

Pigeon-pea wilt has engaged the attention of workers at the Indian Agricultural Research Institute, Delhi, mainly for evolving wilt resistant strains. Indeed it was felt by them that a practical means of fighting the disease could be obtained only in this direction. In Southern India, although red gram wilt has been observed in most of the cultivated areas, there has been no attempt at either estimating the extent of loss due to wilt, or any considerable work done for bringing about a control.

Experimental evidence by many workers has proved that the susceptibility of plants to disease, particularly to the attack of certain vascular pathogens, is conditioned to a considerable extent by their nutrition. In many cases balanced conditions in mineral nutrition has resulted in better vigour of the plant. In certain other cases, the deficiencies of the elements seem to affect the susceptibility of a plant to a disease, while deficiencies of other mineral nutrients have had no apparent effect, thus indicating that

other factors come into play in such cases. It is obvious that these beneficial or harmful effects cannot be easily explained, although their pathological as well as physiological implications are of immense importance.

In the present study, the addition of micro-nutrient elements like boron, manganese and zinc to wilt-sick soils, especially at the levels 20, 40 and 80 p.p.m. resulted in considerable protection to pigeon-pea plants from being attacked by the wilt pathogen. Out of the three elements tried, manganese was seen to be most beneficial; zinc also was satisfactory though not to the same extent as manganese; boron however became injurious after a certain concentration.

The micro-nutrient treated series on the whole yielded better results than the control by lowering the disease incidence to a considerable extent as also promoting plant growth. (vide Text—figs. I and II). It is possible that the additional nutrition in the form of micro-element was utilized mainly by the plant, resulting in better vigour and, therefore, better resistance. The higher dry weights of shoots obtained in the micro-nutrient treatments over those of the control, seemed to indicate this. Manganese, which was seen by the author to yield the best results as regards plant development in infected soil, has been cited as a very essential element for the normal growth of plants in general (McHargue, 1922; 1926, Marsh and Wilbur, 1945; Samuel and Piper, 1929).

The addition of micro-nutrient elements was seen to bring about an effective control irrespective of the season of collection of wilt-sick soil. It was found that soil collected in November was more infective than soil collected in the following March. In this connection mention can be made of the work of some investigators who found a correlation between seasonal variations and activity of soil pathogens. Orton (1908) working on cotton wilt observed that wilt was worse on the whole in wet seasons. Vestal (1946) in observations of economic plant disease fungi and weather at Allahabad, India, found that lowering of the temperature during October created conditions favourable for such root-rotting fungi as *Pythium, Rhizoctonia, Fusarium*, etc. Apparently, the collection and utilization of weather data to the study of activities of soil-borne fungi would help a great deal in evolving new methods for bringing about an effective control.

The addition of minor elements B, Mn and Zn, individually or in combination was seen to benefit the plant even to the extent

of early flowering and production of better quality of seeds which gave a higher germination percentage than those collected from untreated control plants (vide Tables 1-4). The practical value of similar results when obtained in large field scale trials is obvious as the improvement of crops grown in wilt-sick areas will lead to high yields which is very necessary when a large deficit in production in all the provinces has been reported.

The micro-element not only promoted plant growth at the later stage but also stimulated pigeon-pea seeds to early germination, culminating in better seedling vigour and development (vide Table 5). Soaking of pigeon-pea seeds in various concentrations of the three micro-nutrient solutions produced higher germination than the control. The early and better germination induced by the presence of micro-elements, in low concentrations, particularly between 5 and 20 p.p.m should be very useful in the cultivation of pigeon-pea plants, especially from the point of view of emergence when sown in wilt-sick soils, as it has been seen from the pathogenicity trials that a considerable amount of pre-emergence wilt is caused by several of the isolates, including *F. udum* the causal pathogen. The hastening in germination would give the seeds a chance to develop into seedlings and escape fungal attack thus lowering pre-emergence wilt to a very great extent.

It was also noticed that the period of soaking in the nutrient solutions had a decided effect on germination and seedling development. Manganese was most beneficial at all the three levels tried — 20, 40 and 80 p.p.m., the beneficial effect increasing with increase in hours of soaking except in the highest level. Zinc however was beneficial upto 16 hours soaking, while boron affected both germination and seedling development at the 40 and 80 p.p.m. levels when the period of soaking was prolonged. (vide Tables 6 and 7).

The stimulating action of manganese on seed germination and early growth of cultivated plants has been observed by several investigators — Loo and Tang (1945) ; Micheels and Heen (1906); Venkatramani (unpublished). Venkatramani found that manganese sulphate in very high dilutions stimulated the germination of seeds of *Trigonella* and *Hibiscus esculentus* in the laboratory garden soil, when these were soaked for 18 to 24 hours in the solution. Also, with *Trigonella,* small amounts of manganese sulphate added to the spent-up soil increased the fresh weight of the seedlings.

An estimation of the trace-element content of the soil, particularly of the Coimbatore wilt-infested soil, would reveal whether

the improvement seen on addition of boron, manganese and zinc is due to the deficiency of these micro-elements. If so, it can be more definitely stated that the balanced nutrition of the plant has made it resistant to fungal attack.

The behaviour of the fungus in the soil with special reference to its colonization and survival capacity in the presence of boron, manganese and zinc is obviously of unquestioned importance. The experimental data obtained on this point indicated that colonization of *Fusarium* could be controlled to a great extent for at least two to three months duration by the addition of micro-nutrient solutions (20, 40 and 80 p.p.m. levels). Zinc was more efficacious than manganese and boron in preventing *Fusarium* from colonising on host stubble. In the other two treatments, though colonization was poor in the first month, yet a gradual rise in percentage colonization was noted with increasing incubation period (vide Table 8). Subramanian (1950) found that prevention of *Fusarium* colonization on cotton root pieces was not possible under ordinary field conditions. The results obtained above are therefore quite encouraging and could serve as a pointer to the future possibilities in this direction.

The Fusaria in general do not make any appreciable growth in soils. Such being the case, their chances of survival are limited, having to persist as vegetative mycelium, conidia, chlamydospores, sclerotia or saprophytically on organic plant residues. Subramanian (1950) found that the disappearance of *F. vasinfectum* was hastened by modifying suitably the conditions prevailing in the soil. *F. udum* however was found to lack the persistence of the cotton pathogen, *F. vasinfectum,* in soil as it was seen that in survival studies of both the fungi on their respective host stubble, *F. vasinfectum* remained viable up to six months showing 100% survival, while survival of *F. udum* went down after two to three weeks incubation. It is possible that *F. udum* is more shy of microbiological antagonism than *F. vasinfectum.*

The results obtained with survival of *F. udum* (vide Table 10) suggested that disappearance of the fungus from soils could be hastened considerably by the addition of the minor elements — boron, manganese and zinc. Most rapid disappearance was brought about by zinc nutrient solutions while with manganese and boron also survival was not so prolonged as in the control.

These data when viewed in the light of the earlier results obtained with regard to micro-element nutrition and plant vigour suggest that while manganese is essentially important to *Cajanus*

development, the improvement seen with zinc nutrient solutions may be due more to its inhibitory influence on the activity of the fungus in soil. A combined beneficial effect by promoting plant growth and suppressing the fungus is also not impossible in the case of all the elements, particularly with zinc and manganese.

Indeed there is also every possibility of the micro-biological factor governing the behaviour of the fungus, in the presence of modified soil conditions favouring general activity of the soil flora, Hanna and Purvis (1940) found that the total number of micro-organisms in soils was greatly increased by the addition of borax, especially with higher soil reaction-pH 7·7. Plate counts showed that the fungus population was affected more by the addition of borax than was the population of bacteria. The data presented indicated that the "fixation" of boron in limed soils was due, atleast in part, to the increased activity of soil micro-organisms.

Further work on these lines would serve to elucidate the question of survival of *Fusarium*, chiefly in wilt-sick soils, whereby partial control at least could be established.

SUMMARY

1. The addition of micro-nutrient elements — boron, manganese and zinc (especially at levels of 20, 40 and 80 p.p.m.), to wilt-infested soils resulted in greater protection to seedling emergence and in addition promoted plant growth, early flowering and production of better type of seeds with a higher germination capacity.

A beneficial effect was also obtained by presoaking the seeds in various concentrations of the three elements which resulted in inducing early germination and better seedling vigour; the duration of soaking also had an indirect effect on the subsequent emergence and growth of seedlings.

2. The colonization of *Fusaria* on host stubble was temporarily inhibited by the addition of the micro-nutrient solutions. Zinc retarded colonization more than boron and manganese.

3. The survival and persistence of *Fusarium udum* was limited by the presence of micro-nutrient elements in the soil. Zinc hastened the disappearance of the fungus more than boron and manganese.

4. The probable significance of the results obtained are discussed from the point of view of

(a) pigeon-pea wilt control, and
(b) soil-borne diseases of plants.

ACKNOWLEDGMENTS

The author wishes to express her deep gratitude and indebtedness to Prof. T. S. Sadasivan, M.Sc., Ph.D., (London), Director, University Botany Laboratory, Madras, for suggesting the problem and for his continued help, guidance and criticism throughout the course of this investigation and in the preparation of this paper. Her thanks are due to the University of Madras for the award of a research studentship for two years, during the tenure of which, part of the present investigation was carried out.

REFERENCES

Agulhon, H. (1910): Emploi du bore comme engrais catalytique *Compt. rend. Acad. Sci., Paris, 150*: 288-91.

Anon (1935): *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists.* Association of Official Agricultural Chemists, Washington.

Anon (1940): The report of the Imperial Mycologist for 1939-'40. Central Agricultural Research Institute, Pusa, New Delhi.

Arnon, D. I. (1938): Micro-elements in culture-solution experiments with higher plants. *Amer J. Bot., 25*: 322-5.

Bertrand, G. (1927): *Various early references given in Inorganic Plant Poisons and Stimulants, by W. E. Brenchley* (Cambridge University Press), II Edn.

Blair, I. B. (1943): Behaviour of the fungus *Rhizoctonia Solani* Kühn in the soil. *Ann. appl. Biol., 30*: 118-27.

Brenchley, W. E. (1914): The action of certain compounds of Zinc, Arsenic and Boron on the growth of plants. *Ann. Bot., 28*: 283-301.

Brenchley, W. E. and Warington, K. (1927): The role of boron in the growth of plants. *Ann. Bot., 41*: 167-87.

Brown, B. E. (1922): Effect of borax in fertilizer on the growth and yield of potatoes. *U.S. Dept. Agric. Bull.* 988 :8.

Chandler, W. H. (1937); Zinc as a nutrient for plants. *Bot. Gaz., 98*; 625-46.

Conner, S. D. (1917) : The injurious effect of Borax in fertilizers on corn. *Proc. Ind. Acad. Sci.*: 195-199.

Cook, F. C. (1915): Boron: Its absorption and distribution in plants and its effect on growth: *U. S. Dept., Agri. J. Agri. Res.* 5: No. 19; 877-899.

Dennis, R. W. G. (1935-37): A review of developments in the applications of boron in agriculture and horticulture. Boron and plant life — II. *The Fertiliser, Feeding stuffs and Farm Supplies Journal*, p. 1-14.

Eaton, S. V. (1940): Effects of boron deficiency and excess in plants. *Plant Physiol. 15*: 95-107.

Elmer, O. H. (1942): Effect of environment on the prevalence of soil-borne *Rhizoctonia. Phytopath., 32*: 972-77.

Garrett, S. D. (1941): Soil conditions and the take-all disease of wheat, VI. The effect of plant nutrition upon disease resistance. VII. Survival of *Ophiobolus graminis* on the roots of different grasses. *Ann. appl. Biol.,* 28: 14-78; 325-33.

Garrett, S. D. (1944): *Root Disease Fungi.* Chronica Botanica Co. Waltham, Mass. U.S.A.

Hanna, W. J. and Purvis, E. J. (1940): Effect of borax and lime on micro-organisms. *Soil Sci.*, *52*: 275-279.

Haas, A. R. C. (1937): Zinc relation in mottle-leaf of citrus. *Bot. Gaz.*, *98*: 65-86.

Heggeness, H. G. (1942): Effect of boron applications on the incidence of rust on flax. *Plant Physiol.* *17*: 143-4.

Hoagland, D. R. (1944): *Lectures on the inorganic nutrition of plants.* Chronica Botanica Co., Waltham, Mass. U.S.A.

Javillier, M. (1908): Researches sur la présence et le rôle du zinc chez les plantes. (Cited from *Bot. Centralbl.* *110*; 12, 1909.)

Loo, T. L. and Tang, Y. M. (1945): Growth stimulation by manganese sulphate, indole — 3 — acetic acid and colchicine in the seed germination and early growth of several cultivated plants. *Amer. J. Bot.*, *32*: 106-114.

Marsh, A. W. and Wilbur, L. P. (1945): Responses of plants to additions of manganese to some Oregon soils. *Amer. Soc. Agron.*, *37*: 1-8.

Mazé, P. (1914): Influence respectives des elementes de la solution minérale sur le developpement du mais. *Ann. Inst. Pasteur*, *28*: 21-68. (Cited from Mazé, 1915).

Mazé, P. (1915): Determination des elements minéraux rares necessaires au developpement du mais. *Compt. rend. Acad. Sci. Paris*, *160*: 211-214.

Mazé, P. (1919): Reserche d'une solution purement minérale capable d'assurer l'évolution complète du maïs cultivé à l'abri des microbes, *Ann. Inst. Pasteur*, *33*: 139-173. (Cited from Warington, 1923).

McHargue, J. S. (1919): The effect of manganese on the growth of wheat: A source of manganese for agricultural purposes. *J. Ind. Eng. Chem.* *11*: 332-335.

McHargue, J. S. (1921): The occurrence and distribution of manganese in plants. *Ky. Sta. Rept.* 36-37.

McHargue, J. S. (1922): The role of manganese in plants. *J. Amer. Chem. Soc.*, *44*: 1592-8.

McHargue, J. S. (1923): Effect of different concentrations of manganese sulphate on the growth of plants in acid and neutral soils and the necessity of manganese as a plant nutrient. *J. agr. Res.*, *24*; 781-795.

McHargue, J. S. (1925): The occurrence of copper, manganese, zinc, nickel and cobalt in soils, plants and their possible functions as vital factors. *J. agr. Res.*, *30*: 193-196.

McHargue, J. S. (1926): Manganese and plant growth. *J. Indust. Engng. Chem.* *18*: 172.

Micheels, H. and Heen, P. de (1906): The stimulation action of manganese on germination. *Bul. Acad. Roy. Belg. Cl. Sci.*, 5: 288-289. (Cited from *Bot. Centralbl.*, *102*: 330, 1906).

Millikan, C. R. (1938): A preliminary note on the relation of zinc to disease in cereals. *J. Dept. Agric.* Vict. *36*: 409-16.

Millikan, C. R. (1942): Studies on soil conditions in relation to root-rot of cereals. *Proc. Roy. Soc. Vict.*, N.S. 54: 145-95.

Nowell, W. (1923): *Diseases of crop plants in the lesser Antilles.* London, W. Ind. Committee. (Cited from Garrett, 1944).

Orton, W. A. (1908): Cotton wilt. *U. S. Dept. Agric. Bull.* 333: 1-24.

Padwick, G. W. (1940): The genus *Fusarium* — *V. Fusarium udum* Butler, *F. vasinfectum* Atk. and *F. lateritium* Nees var. *uncinatum* Wr. *Ind. J. agric. Sci.*, *10*: 863-873.

Pryor, D. E. (1940): The effect of some mineral nutrients on the development of club rot of crucifers. *J. agri. Res.* *61*: 149-160.

Radha, K. (1948): Soil conditions and wilt diseases in plants with special reference to *Rhizoctonia bataticola* (unpublished).

Rawlins, T. E. (1933): *Phytopathological and Botanical Research Methods*, John Wiley, London.

Sadasivan, T. S. (1939): Succession of fungi decomposing wheat straw in different soils, with special reference to *Fusarium culmorum*. *Ann. appl. Biol. 26*: 497-508.

Samuel, G. and Piper, C. S. (1929): Manganese as an essential element for plant growth. *Ann. appl. Biol. 16*: 494-524.

Shear, G. M. and Wingard, S. A. (1944): Some ways by which nutrition may affect severity of disease in plants. *Phytopath. 34*: 603-605.

Smith, P. G. and Walker, J. G. (1941): Certain environal and nutritional factors affecting *Aphanomyces* root rot of garden pea. *J. agri. Res. 63*: 1-20.

Sommer, A. L. and Lipman, C. B. (1926): Evidence on the indispensable nature of zinc and boron for higher green plants. *Plant Physiol.* 1. 231-249.

Subramanian, C. V. (1946a): Some factors affecting the growth and survival of *Fusarium vasinfectum* Atk. the cotton wilt pathogen in the soil, with special reference to micro-biological antagonism. *Jour. Ind. Bot. Soc. 25*: 89-101.

Subramanian, C. V. (1946b): The saprophytic activity of *Fusarium vasinfectum*, the cotton wilt pathogen in the soil. 1. Colonisation of cotton roots buried in the soil. *M. O. P. Iyengar Commemoration Volume, Jour. Ind. Bot. Soc. 26*: 209-213.

Subramanian, C. V. (1950): Soil conditions and wilt diseases in plants with special reference to *Fusarium vasinfectum* on cotton. *Proc. Indian Acad. Sci., 31*: 67-102.

Sulochana, C. B. (1948): Soil conditions and wilt diseases in plants, with special reference to colonisation and survival of *Fusaria* in soils treated with micro-elements, (unpublished).

Venkatramani, K. S. (1948): Studies on Indian vegetables. (Thesis approved for the degree of Doctor of Philosophy, University of Madras). (unpublished).

Vestal, E. F. (1946): Observations on economic plant disease fungi and weather at Allahabad, India, during the 1945-46 crop seasons. *Pl. Disease Reporter, 30*: 8.

Walker, A. G. (1941): The colonisation of buried wheat straw by soil fungi, with special reference to *Fusarium culmorum*. *Ann. appl. Biol. 28*: 333-50.

Warington, K. (1923): The effect of boric acid and borax on the broad bean and certain other plants. *Ann. Bot. 37*: 629-672.

Warington, K. (1925): The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. *Ann. Bot. 40*: 27-42.

Wingard, S. A. (1941): The nature of disease resistance in plants, I. *Bot. Rev. 7*: 59-109.

Wolfe, B. (1940): Factors influencing availability of boron in soil and its distribution in plants. *Soil Sci. 50*: 209-216.

Yogeswari, L. (1948): Trace element nutrition of fungi I. The effect of Boron, Zinc and Manganese, on *Fusarium* species. *Proc. Indian Acad. Sci. 28*: 177-201.

Young, V. H. (1938): Control of cotton wilt and "rust", or potash hunger, by the use of potash containing fertilizers. *Bull. Ark. Agric. Expt. Sta.* 358.

Zachariah, A. (1949): Micro-ecology of cultivated fields of South India with special reference to the occurrence and physiology of *Fusarium*. (Thesis approved for the Degree of Doctor of Philosophy, University of Madras). (unpublished).

Explanation of Plates

PLATE I: Micro-element nutrition and development of red gram plants.

Figures 1 and 2: Manganese and boron nutrient treatments at 20, 40 and 80 p.p.m. levels. Controls are on the extreme left.

Figures 3 and 4: Zinc and "combination" nutrient treatments at the 20, 40 and 80 p.p.m. levels. Controls are at the extreme left.

PLATE II: Boron injury in *Cajanus* leaves.

Figures 5 and 6: Boron concentration at 80 p.p.m. level.

Figure 7. Boron injury in leaves of individual boron and "combination" treatment. a: individual boron treatment; b: combination (B + Mn + Zn); C: Control.

PLATE III. Figures 8, 9 and 10. Development of seedlings from seeds soaked in various concentrations of boron nutrient solution.

a = 0·5 p.p.m.; b = 1 p.p.m.; c = 2 p.p.m.; d = 5 p.p.m.; e = 20 p.p.m.: f = 40 p.p.m.; g = 80 p.p.m.; h = 160 p.p.m.; i = 320 p.p.m.

LARVAL STAGES OF SOME COPEPODS IN THE MADRAS PLANKTON AND THEIR SEASONAL FLUCTUATION

BY

S. KRISHNASWAMY, B.Sc.

(Zoology Laboratory, University of Madras)

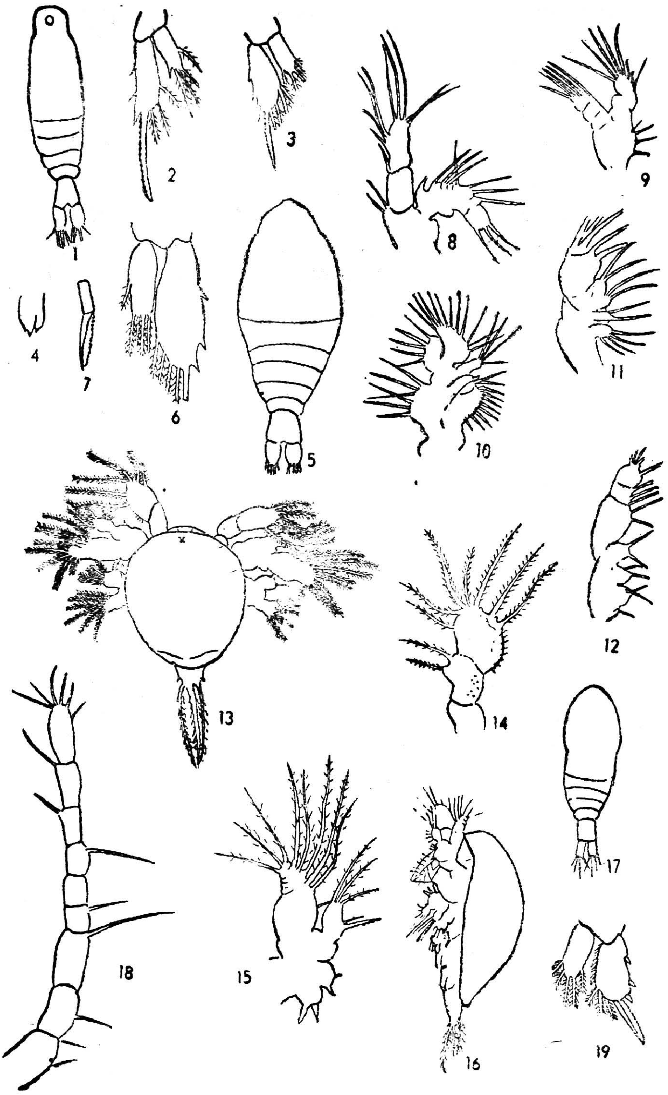
Introduction

Though the development of the copepods had attracted the notice of many like Claus (1876), Marsh (1900), Oberg (1906), Grandori (1912), Dietritch (1915), Lebour (1916), Chappuis (1916), Murphy (1923), Brian (1925), Gurney (1929, 30), Nicholls (1934, 41) and Johnson (1934, 48), our knowledge of the larval stages of copepods is far from complete. The need for detailed study of the developmental stages of copepods cannot be over-emphasised as "probably without it we shall never reach a satisfactory classification of the copepoda" (Gurney, 1930). In the present paper, the nauplii and copepodites occurring in the Madras inshore plankton are identified, sketched and described fully. Wherever one or the other of the larval stages of allied species have been described by previous authors, tabular comparisons have been added. As the numerical abundance of these larval stages indicate the seasonal reproductive activity of the copepods and has a direct bearing on the fluctuation in copepod population, this aspect of the occurrence in the copepod larvae has also been studied.

Material and Methods

Only inshore plankton was examined. The females with eggs in the brood-sacs were transferred from the plankton into small dishes containing fresh sea water. Normally the eggs hatched out in twelve hours and the nauplii were thus identified and sketched in the living condition. All attempts to rear the nauplii into copepodite stages failed. The copepodite stages were however taken directly and identified as such or reared till the generic and specific characters became well defined.

In the present paper, the larval stages of the following forms are described: (1) *Centropages furcatus,* (2) *C. tenuiremis,* (3) *Pseudodiaptomous aurivilli,* (4) *Schmackeria serricaudatus,*


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

(5) *S. annandalei,* (6) *Temora sp.,* (7) *Labidocera sp.,* (8) *Acartia erythraea,* (9) *Tortanus gracilis,* (10) *Longipedia sp.,* (11) *Macrosetella gracilis,* (12) *Miracia efferata,* (13) *Euterpina acutifrons,* (14) *Oithona rigida,* (15) *Pseudanthessius maximus,* (16) *Corycaeus sp.*

DESCRIPTION OF THE LARVAE

Family: *Centropagidae*

Centropages furcatus Dana.

Occurrence: The early copepodites of Centropages furcatus are common in the plankton in the month of March. A few stray specimens have been taken in July also. The adults are found from January to March in the plankton.

Size: Length 0·525 mm. (first stage) Fig. 1.

Colour: The copepodite is transparent and colourless. The eye is of a deep red colour and capable of rotatory movement as in adult.

Body: The copepodite resembles the adult in general shape. The projection on the posterior corner of the metasome, characteristic of this species is absent. Urosome single jointed. The furcal ramus longer than broad and carries four terminal setae and a bristle. The setae are generally broken at some length or other and never complete.

Appendages: *Antennule:* Eight jointed and carries a number of setae especially at the apex. *Antenna* and mouth parts as in the adult.

First swimming feet: Biramous and each ramus is single jointed. The exopodite which is nearly twice as long as the endopodite has four outer, lateral short spines, one long, terminal, serrate spine, and four inner setae. The endopodite carries five setae (Fig. 2). *Second swimming feet:* The rami long and narrow, longer than those of the first pair. The exopodite carries three, short, lateral spines on the outer margin, one long, serrate, apical spine, and three inner setae. The endopodite which is half as long as the exopodite carries four setae (Fig. 3). *Third swimming feet* is rudimentary (Fig. 4).

Centropagus tenuiremis: Thompson and Scott (Fig. 5).

Occurrence: The copepodites of this are very common in the February-March plankton. They occur in September and December also.

Colour: In the living condition, the larval is transparent and colourless.

Size: Length 0·606 mm.

Body: The anterior end of the cephalosome is pointed as in the adult. The eye is prominent. The posterior corners of the metasome rounded and not produced into acute processes as in the adult. The abdomen is single jointed. The furcal ramus is as long as broad and carries five setae.

Appendages: The *antennule* is eleven jointed, the third, fourth, seventh, eighth and the eleventh joints carry an 'aesthete' each. The mouth parts are as shown in the figures 8, 9, 10, 11 and 12. *First swimming feet*: Biramous, each ramus single jointed. The exopodite which is broad and long carries three, outer, short lateral spines, one long apical, serrate spine, and four inner setae. The endopodite which is only a little more than half as long as exopodite carries four apical and one lateral seta. *The second swimming feet*: resembles the first one. *The third swimming feet* is rudimentary. The terminal spines in the swimming feet appear to be very fragile. They were never complete in most of the specimens.

Remarks: Gurney (1930) figures the nauplius of *C. hamatus* and has commented on its development. But as he has not described the copepodites or its development, a comparison is not possible.

Family: *Pseudodiaptomidae*

Pseudodiaptomous aurivilli Cleve.

Occurrence: The naupliar stages of this copepod are common in the plankton in July and December. Several females with eggs were reared in the laboratory and an account of the first nauplius is given here.

Nauplius: 1st stage (Fig. 13).

Colour: The nauplius is colourless when it is hatched.

Size: Length 0·175 mm. (without the setae on the posterior side).

Body: The nauplius is more or less rounded. The posterior end is short, only one fourth the length of the anterior end. Labrum is large and fringed with setae. Eye red in colour. There are three unequal, plumose setae on the posterior end of the nauplius.

Appendages: The *antennule* is of the usual shape, three jointed and flattened. The second joint bears two setae on its outer side while the terminal joint which is very flattened has its inner margin hirsute and carries seven plumose setae (Fig. 14). The *antenna* is biramous. The coxopodite and the basipodite bear two short masticatory spines each on the inner side. The single jointed endopodite carries two inner lateral spines, and three apical setae. The exopodite is indistinctly six jointed and carries five inner laternal and three terminal setae (Fig. 15). The *mandible* has a coxopodite with a single short inner spine, a basipodite with three inner spines and a biramous palp. The single jointed endopodite is armed with six lateral and three terminal setae. The exopodite bears three lateral and three terminal setae.

Schmackeria annandalei (Sewell).

Occurrence: The plankton from Cooum, especially in the month of May is very rich in nauplii and copepodites of *S. annandalei.* A short description of the nauplius as well as the first copepodite is given here.

Nauplius (Stage III ?) (Fig. 16).

Size: Length 0·175 mm.

Colour: In the living condition the nauplius is deep yellow in colour. The eye is red in colour.

Body: The nauplius is longer than broad and is of the usual *Calanoid* type. The posterior region bears three unequal plumose spines. The labrum is large and carries a number of short, stiff, bristles on it.

Appendages: The *antennule* is three jointed, the joints being flattened. The terminal joint carries eight setae. The *antenna* is biramous. The coxopodite and the basipodite carry 'masticatory hooks'. The single jointed endopodite has four short inner and three apical setae. The exopodite which is indistinctly four jointed carries seven setae. The *mandible* has a coxopodite and a basipodite which carry short, thick spines. The palp is biramous. The endopodite is short single jointed, carrying three apical and three lateral setae. The exopodite which is indistinctly four jointed carries four setae. *Maxillae*: *rudimentary.*

Copepodite Stage I (Fig. 17).

Size: 0·45 mm.

Colour: In the living condition, it is coloured yellow.

Body: The cephalothorax is elliptical in shape and is slightly bulged about the middle of the cephalosome. The lateral projec-

tions on the genital segment which is characteristic of this species is absent. The abdomen is single jointed. The furcal rami are longer than broad and carry four setae each.

Appendages: The *antennule* is nine jointed. The number of setae and their arrangement as shown in the figure (Fig. 18). The *antenna* is biramous. The mouth parts as in the adult. *First swimming feet*: Biramous, both the rami being single jointed. The exopodite which is slightly longer and broader than the endopodite carries three, short, lateral, spines, one long serrate apical spine and three inner setae. The endopodite has five terminal setae. The inner margin of the exopodite is hirsute (Fig. 19). *The second swimming feet* larger than the first, but the armature as in the first. *The third swimming feet* is rudimentary.

Schmackeria serricaudatus (Scott).

Occurrence: The copepodites of this copepod are common in the plankton collected in July though they occur throughout the year.

Copepodite I (Fig. 20).

Size: Length: 0·4 mm.

Colour: Colourless and transparent or of a light yellow colour. Dark brown or black spots are found in the cephalothorax. Eye dark red in colour.

Body: The cephalothorax is more or less elliptical in shape. The serrations on the last thoracic segment, found in the adult are abesnt. The abdomen is two jointed. Caudal furca, longer than broad, and each ramus carries four setae.

Appendages: The *antennule* is eight jointed and each joint carries one or two setae. The *antenna* and mouth parts as in adult. There are three pairs of swimming feet. *First swimming feet*: The exopod as well as endopod are single jointed. The exopod carries three outer serrate spines, one apical, long serrate spine, which is broken in most of the specimens, and three inner setae. The endopod is as long as the exopod and has four setae (Fig. 21). *Second swimming feet*: The single jointed exopod and endopod are long and broad. The exopod carries three short, serrate, outer spines, a long serrate apical spine, and three inner setae. The endopod carries four setae and it is slightly shorter than the exopod. (Fig. 22). *Third swimming feet* rudimentary.

Remarks: *S. serricaudatus* is found to breed throughout the year.

Family: *Termoridae*

Temora sp. **(Fig. 23).**

Occurrence: Large numbers of this were present in the plankton collected in November-December. In the living condition, the larva is colourless.

Size: Length 0·52 mm.

Body: The cephalosome which is rounded anteriorly is laterally produced into wing like expansion, characteristic of the copepodites of *Temora*. The cephalosome is nearly as long as the metasome. The urosome is very short. The furcal ramus is long and carries three short spines and a long seta.

Appendages: The *antennule* is eight jointed. The *antenna* is biramous, with a two jointed endopodite and an indistinctly six jointed exopodite (Fig. 24). Mouth parts as shown in the figure (Figs. 25, 26, 27, 28). *The first swimming feet* is biramous, with a single jointed exopod and an endopod. The exopod which is twice the length of the endopod carries, three, short, outer spines, one terminal, serrate, spine, and three inner setae. The endopodite carries three apical, and one lateral seta (Fig. 29).

Remarks: As adults of both *T. turbinata* and *T. discaudata* occur in the plankton, these copepodites though undoubtedly belonging to this genus, cannot be identified specifically.

Family: *Pontellidae*

LABIDOCERA SP.

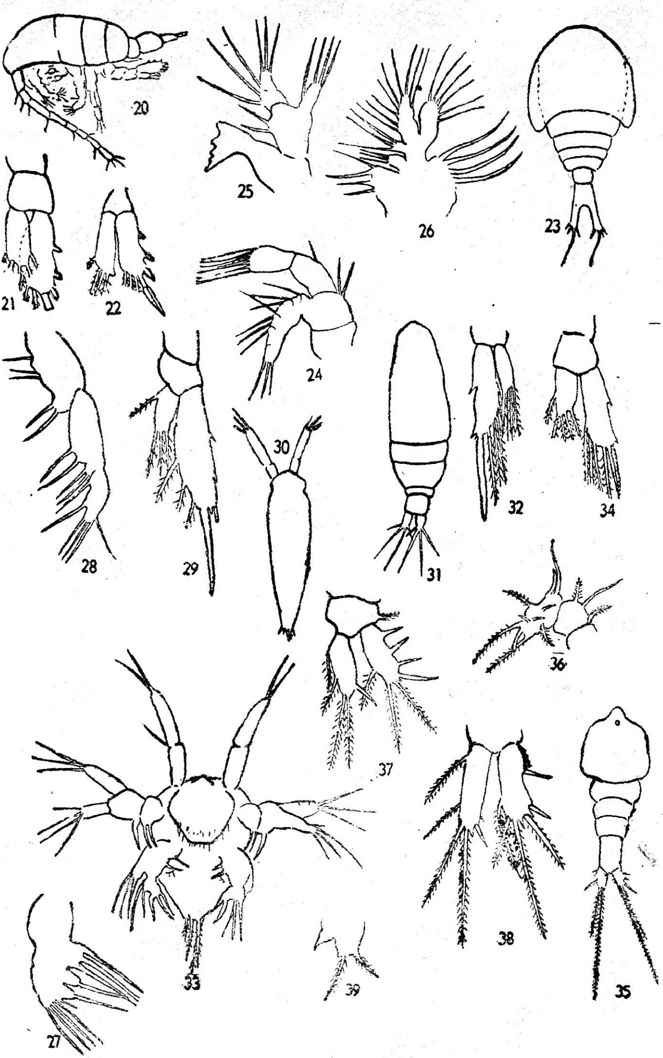
Nauplius (Stage II ?): (Fig. 30).

Occurrence: The nauplii of this species are common from September till March.

Size: Length 0·6 mm.

Colour and Body: In the living condition the nauplius is yellow in colour. The body is long and oval with the anterior end slightly broader and the posterior end which is tapered carrying four, short, unequal, spines. Labrum is long and prominent.

Appendages: The *antennule* is three jointed and the terminal joint carries three setae. The *antenna* is biramous. *Coxopodite* and *basipodite* with a spine each on the inner side. The endopod is single segmented and carries two terminal setae while the exopodite, is indistinctly six jointed and carries five setae. *Mandible* with a biramous palp. The single jointed endopodite bears


20
25
26
23
21
22
24
28
29
30
31
32
34
36
37
33
38
35
39
27

two plumose spines, while, the exopodite which is indistinctly four jointed, carries three setae.

Remarks: As adults of a number of species of *Labidocera* occur in the plankton, these nauplii though undoubtedly belonging to this genus cannot be identified specifically.

Family: *Acartidae*

Acartia erythraea Giesbrecht. (Fig. 31).

Occurrence: The early copepodites of this species are present in the plankton almost throughout the year and indicate a continuous breeding reaching their numerical peaks in February and March. In the living condition, the body of the larva, is transparent and colourless. The eye, is coloured red.

Body: The larva is 0·5 mm. long and resembles the adult in the general shape of the body. The urosome is however, single jointed. The spines on the posterior corner of the metasome, present in the adult are absent. The furcal rami are twice as long as broad and tipped with three setae and a short bristle each.

Appendages: The *antennule* is eight jointed. The *antenna* and mouth parts as in the adult. The *first swimming feet* biramous with a single jointed, exopodite and endopodite. The exopodite which is nearly twice the length of the endopodite has two short lateral spines, one long terminal serrate spine, and five inner setae. The endopodite carries four setae. (Fig. 32). The *second swimming feet* resembles the first one, except for the larger size. The *third swimming feet* is rudimentary.

Family: *Tortanidae*

Tortanus gracilis (Brady).

Occurrence: The nauplii and copepodites of this are present in the plankton from July to December, but common in July.

The nauplius (Stage III ?) (Fig. 33).

Colour: The nauplius is colourless. The base of the appendages are however deep yellow in colour. Eye is red in colour.

Size: Length 0·36 mm.

Body: The body is oval in shape, the anterior end being slightly broader than the posterior end and the posterior end carry-

ing three long and a short plumose seta and two short spines. Labrum, large and armed with short bristles.

Appendages: The *antennule*, which is indistinctly three jointed has a seta on the second joint and three setae at the terminal joint. The biramous *antenna* has a coxopodite which carries two, curved, masticatory spines, a basipod with a single spine on the inner side, a single jointed endopodite with four terminal setae and an indistinctly four jointed exopodite with six setae. *Mandible*: Coxopodite with one and basipodite with two masticatory hooks. Endopodite, two segmented with three thick spines and three terminal setae. The four jointed exopodite carries four long setae. *Maxilla*: rudimentary.

Copepodite Stage I.

Size: Length 0·65 mm.

Colour: Varying shades of yellow pigments present in the body of the animal. In the living condition, the larva can be easily recognised by the long, curved, yellow coloured, setae of the *antenna* which extend upto the posterior end of the cephalothorax.

Body: The cephalosome is truncate towards the anterior side. The metasome is composed of four segments. Urosome single jointed. Caudal rami, symmetrical, and each ramus is armed with three long setae and a short bristle. Labrum is fringed with a number of short, stiff, hairs. Eye present as a dark red spot.

Appendages: The *antennule* is eight jointed, the terminal joint carrying six setae. The *antenna* is biramous. The endopodite is two jointed and the second joint carrying four terminal setae. The four jointed exopod carries four terminal setae and a small bristle.

Swimming feet: There are two pairs of biramous swimming feet. A third pair which is rudimentary is also present. *First swimming feet*: The single jointed exopodite carries two small outer spines, a long serrate, terminal spine and four inner setae. The endopodite, which is only half as long as the exopodite, carries four terminal and one inner lateral seta (Fig. 34). *Second swimming feet* is similar to the first except for the rami being slightly longer. *Third swimming feet* is rudimentary.

Remarks: The life history of *Tortanus discaudatus* has been studied by Johnson (1934) and a comparison of *T. gracilis* with that form is interesting.

NAUPLIUS

	Tortanus discaudatus	Tortanus gracilis
Length	0·23 — 0·25 mm.	0·36 mm.
Antennule	Second joint with 3 and the terminal joint with 4 setae.	Second joint with a single seta and the terminal joint with 3 setae.
Antenna	Endopodite with 6 setae. Exopodite carries 7 setae.	Endopodite with 4 terminal setae. Exopodite with 6 setae.
Caudal spines	6, uneven in length	6, uneven in length.

COPEPODITE

	Tortanus discaudatus	Tortanus gracilis
Length	0·55 mm.	0·65 mm.
Antennule	Eight jointed	Eight jointed
First swimming feet	Exopodite with two spines and four setae. Endopodite with seven setae.	Exopodite with three spines and four setae. Endopodite with five setae.

Sub-Order Harpacticoida

Family : *Longipediidae*

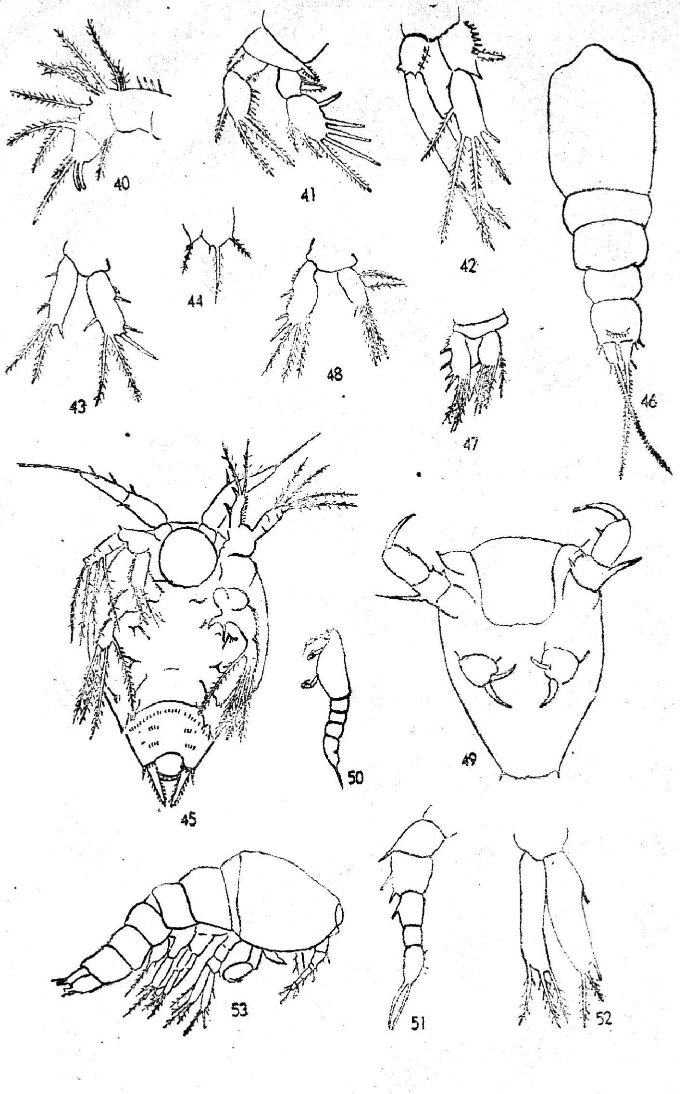
Longipedia sp.

Occurrence : Several first and second copepodites of this copepod were taken in the month of July. In the living condition, the larvae are green in colour.

Copepodite I (Fig. 35).

Size : Length 0·45 m.m.

Body : The body consists of five segments, excluding the furcal ramus. The cephalosome is drawn out into a knob-like projection on the anterior side, while its lateral margins are produced into wing-like projections in some specimens. The thoracic seg-


40
41
42
43
44
48
47
46
45
50
49
53
51
52

ments decrease in length gradually. The single jointed abdomen is longer than broad. The caudal furca is as long as broad and carries three outer lateral spines a long inner and a short outer plumose seta.

Appendages: The *antennule* is three jointed. The second joint bears two plumose and a naked spine while the terminal joint has six plumose and four naked spines. (Fig. 36). The *antenna* and the mouth parts are well developed and of the adult form. *The first swimming feet* is biramous. The basipodite carries a plumose spine on the inner as well as the outer side. The exopodite is unjointed and carries five outer spines which are naked, and a short inner lateral, and a terminal, long, plumose seta. The exopodite is armed with teeth towards outer margin. The endopodite is a little shorter than the exopodite and it carries four plumose setae and a short naked seta towards the outer side. The endopodite bears four teeth towards the distal margin on the outer side (Fig. 37). *The second swimming feet* is also biramous. The endopod is only a little longer than the exopod. The exopodite has its proximal, outer margin armed with four teeth and carries three outer lateral spines, a short, inner plumose seta, and two long, terminal plumose setae. The endopodite is more slender than the exopodite and it carries two short spines on the outer side and two plumose setae on the inner side and two terminally (Fig. 38). *The third swimming feet* is rudimentary. The exopodite is represented by a papilla with two plumose setae while, the endopod by a single seta (Fig. 39).

Copepodite II.

Size: Length 0·50 m.m.

Body: General shape as in Copepodite I. The body shows an increase in the number of segments. There are four pairs of swimming feet, the fourth pair being rudimentary. The endopod of the second swimming feet assumes its characteristic shape.

Appendages: *Antennule* is indistinctly four jointed. The second joint is armed with teeth on the outer side and two plumose setae, the outer one being twice the length of the inner one. The third joint bears seven plumose spines and an 'aesthete'. The terminal joint carries two plumose spines and two 'aesthetes' (Fig. 40).

The first swimming feet: The basipodite bears a short plumose spine on the inner as well as the outer margin. The exopodite is two jointed and is longer than the endopodite. The first exopod joint is armed with teeth on the outer side, a lateral plumose spine

on the outer side while the second joint bears one outer lateral spine, four long plumose setae and two short inner setae. The first joint of the endopod is armed with teeth on the inner side. The second joint has three plumose setae and a plumose spine and on its outer margin carries a number of teeth (Fig. 41). *Second swimming feet* biramous, both the rami being two jointed. The first exopodite joint has its outer lateral margin drawn out and carries a plumose spine and is armed with a number of teeth. The second joint bears one outer spine and three terminal and one inner lateral plumose seta. The endopod is longer than the exopod. The first joint bears a number of teeth on the outer side. The second joint, which is four times the length of the first one has three short terminal and one lateral, plumose spine (Fig. 42). *Third swimming feet*: Exopodite as well as endopodite one jointed. Exopodite has two outer spines and three apical plumose setae and an inner plumose seta. The endopodite with one outer spine and three inner plumose setae (Fig. 43). *Fourth swimming feet* rudimentary (Fig. 44).

Remarks: Gurney (1930) has described the copepodites of *Longipedia sp.* Later Nicholls (1934) has completed the life history. Gurney (loc. cit) referred to his forms as 'pink' and 'green' form. A comparison of the form described by Gurney with the present form is interesting.

	Gurney's 'green form'	Madras form
Length	0·45 mm.	0·45 mm.
First leg	Endopod with two long plumose and two short setae.	Endopod with four plumose setae and one outer naked seta.
	Exopod with four lateral two terminal and one inner seta.	Exopod with four lateral two terminal and one inner seta.
Second leg	Exopod with three lateral spines two terminal and one inner seta.	Exopod as in Gurney's form.
	Endopod with two terminal two lateral and one inner seta.	Endopod with two terminal, two lateral setae and two outer spines.

Gurney's pink form (II Copepodite stage) is similar to the Madras form in all respects except the second leg, the chief points of difference are indicated below.

	Gurney's 'Pink form'	Madras form
II Copepodite Stage Second leg	*Exopod*	
	The inner edge of the first joint hirsute, outer side bears one spine.	The inner edge of first joint not hirsute. Outer edge carries a spine and several teeth.
	Second joint with one outer spine three terminal setae and one inner spine.	Same as in Gurney's form.
	Endopod	
	First joint with one spine.	First joint bears three on the outer side.
	Second joint with two lateral and three terminal spines.	Second joint with one lateral and three terminal spines.

Remarks: Wilson (1932) states that "it sticks rather closely to the bottom where the long second legs assist it in locomotion". This is probably the reason for the absence of the adults in the plankton.

Family: *Tachidiidae*

Euterpina acutifrons (Dana).

Occurrence: The nauplii and copepodites of this harpacticoid are present in the plankton throughout the year. They are however very common in July, September, December-January and March.

Nauplius (6th stage). (Fig. 45).

Length 0·3 m.m.

Colour: Transparent and colourless.

Body: The nauplius is oval in shape, and is slightly longer than broad. The posterior region is armed with two long stout and two short slender spines. The labrum is prominent.

Appendages: *Antennule* is three jointed. The second joint is nearly three times longer than the first, and has two short setae. The third joint is as long as the second and has two outer-lateral setae and a long terminal seta. The *antenna* is biramous. The endopod is single jointed and has its outer margin hirsute. It carries two short and two long setae. The exopod is indistinctly four jointed and carries four setae. *Mandible*: The endopod with two claw-like spines on the inner side. The exopod is indistinctly four jointed and carries four plumose setae. The *maxillae* and *swimming feet*: rudimentary.

Copepodite I. (Fig. 46).

Length 0·35 m.m.

Colour: Transparent and colourless or slightly greenish. Eye red in colour.

Body: Resembles the adult in general shape. The body is composed of five joints, including the cephalosome. The anterior end of cephalosome produced. The urosome is two jointed and there is a semi-circular fringe of 'hairs' on the second joint. The furcal rami are longer than broad and carry three short outer bristles and a long, inner plumose seta each. *Appendages*: *Antennule* is four jointed. The first joint is very small, the second joint which is very long and stout carries three setae, the third joint which is about ⅓ the length of the second has two setae, and the terminal joint, which is as long as the third joint, carries three setae. The *antenna* consists of a two jointed endopod and a single jointed exopod. *Mandible* and *Maxillae* as in the adult. *First swimming feet*: Both the rami are single jointed. Exopod carries four lateral spines and three setae. Endopod, which is slightly smaller than the exopod, carries five setae and a spine (Fig. 47). *Second swimming feet*: Exopod has its outer margin denticulate and carries three spines and three setae. Endopod is shorter than the exopod and has a spine and five setae (Fig. 48).

Family: *Macrosetellidae*

Macrosetella gracilis (Dana).

Occurrence: The nauplii and early copepodites are a regular feature in the plankton from August till January, reaching a maximum in September, October months. The peculiar habit of the larvae to cling to *Trichodesmium* strands in the plankton has already been reported by the author (1948).

Nauplius Stage II. (Fig. 49).

Size : Length 0·129 m.m.

Body : The nauplius is more or less triangular in shape. Red pigment is present inside the body. The eye appears as a red spot. Labrum prominent. There are two short posterior spines. *Appendages* : The *antennule* is rudimentary, with one long apical seta. *Antenna* prehensile, one jointed. The endopod is single jointed and is armed with a claw. The exopod is represented by a spinous projection on the basipodite. The *mandible,* represented by a single lobe with two curved setae.

Copepodite, Stage I. (Fig. 50).

Length 0·484 m.m.

Body : The body is composed of five segments including the cephalosome. Eye is distinct.

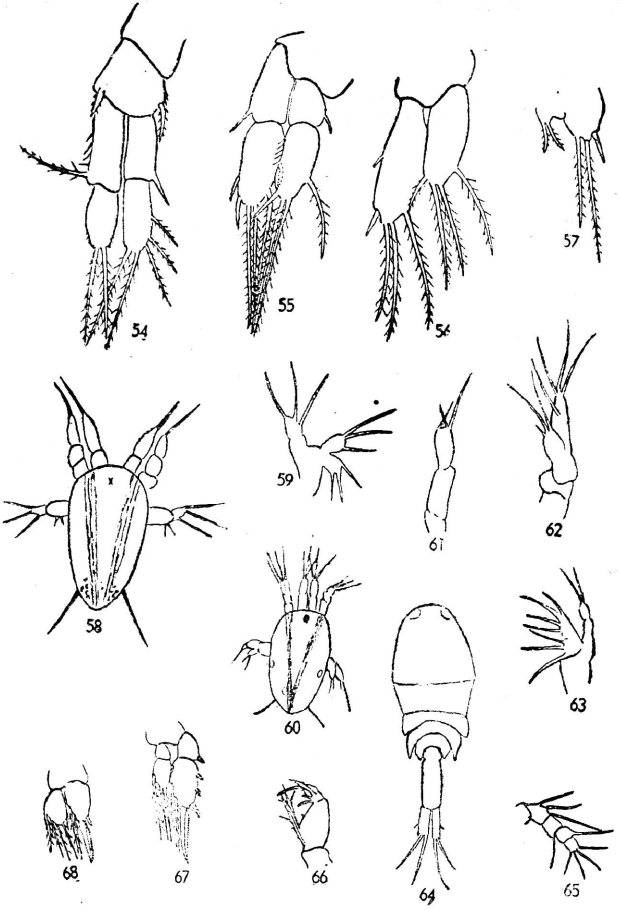
Appendages : The *antennule* is five jointed. The outer margin of the first joint is produced into a spinous process. The second joint bears two setae, third one, one seta and the fifth, two apical setae. (Fig. 51). *Antenna* two jointed with three apical setae, the middle one being the longest. *Mandible* : simple, with a pointed tooth. Palp absent. *Maxillae* : rudimentary. *Maxilliped* : prehenslie, two jointed. The terminal joint bears a claw. *First swimming feet* : biramous, with an unjointed exopodite and endopodite. The endopodite carries two apical and one sub-apical setae and the exopodite two apical and two short sub-apical setae. (Fig. 52). *Second swimming feet* : indistinctly biramous, the outer lobe with a spine and two long apical setae and the inner lobe with two setae.

Miracia efferata Dana. (Fig. 53).

Occurrence : Four copepodites (Stage II) of this were present in the plankton collected in March 1949. The animal is of a deep red colour in the living condition. Formalin fixed ones are yellow in colour.

Size : Length 0·75 m.m.

Body : The body is composed of six segments including the cephalosome. There are two prominent corneal lenses on the anterior end of the cephalosome as in the adult. The metasome as well as the urosome segments decrease in breadth gradually and are laterally compressed. The furcal ramus is as long as broad and carries two short spines and a long seta. There are four pairs of swimming feet.


54
55
56
57
58
59
61
62
60
63
68
67
66
64
65

Appendages: *Antennule* is five jointed. *Antenna* and the mouth parts as in the adult. *First swimming feet*: The basipodite carries a spine each towards the outer and inner side. The exopodite is two jointed. The first joint bears an outer lateral spine, the second joint and five plumose spines. The endopodite is also two jointed. The first endopod joint bears a number of stiff bristles on the inner side and a seta. The second joint has its outer and inner margin armed with teeth towards the distal side and bears two terminal plumose setae. (Fig. 54) *Second swimming feet*: biramous with the two jointed exopodite and endopodite. The first exopod joint with one outer spine, while the second joint has two outer spines, two terminal and two inner plumose setae. The first endopod joint has a single spine while the terminal joint has four plumose spines. (Fig. 55). The third and the fourth swimming feet as shown in the Figures 56 and 57.

Remarks: The adults of *M. efferata* have been recorded only twice in Madras, in July and in March.

SUB-ORDER: CYCLOPOIDA

Family: *Oithonidae*

Oithona rigida Giesbrecht. (Fig. 58).

Nauplius 1st stage: The general appearance is like that of any cyclopoid nauplius.

Colour: In the living condition the nauplius is light yellow in colour. Eye present as a red spot.

Size: Length 120 μ without the posterior setae and 125 μ with the setae.

Body: The nauplius is longer than broad, the anterior end being broader and tapering posteriorly. The labrum is large.

Appendages: The *antennule* is three jointed and the second joint carries a short seta on its inner side and the third two long lateral and a short apical seta. The *antenna* is biramous. The exopod is six jointed the joints are however very indistinct and could be made out only with difficulty. The exopod carries five lateral and one apical seta. The endopod which is unsegmented carries two stout apical and the two lateral setae. *Mandible*: The exopodite is two jointed and the terminal joint bears three setae. The endopodite is also two jointed and each joint bears three setae. (Fig. 59).

Remarks: Gibbons and Ogilvie (1933) have studied the life history of *Oithona helgolandica* and *O. spinirostris*. The following table gives a comparison of *O. helgolandica* and *O. rigida*.

	O. helgolandica	O. rigida
Length	0·115 mm.	0·120 mm.
Posterior spines	4	2
Antennule	Three jointed. 2nd joint with 3 and 3rd joint with 4 setae.	Three jointed. 2nd joint with 1 seta and the third with 2 setae.
Antenna	Coxopodite with masticatory processes. Exopod 6 jointed with 6 setae.	Exopod 6 jointed with 6 setae.
Mandible	Coxopodite with bristles. Endopod in two joints the first of which is short. The 2nd joint carries 4 setae. Exopod 4 jointed, 4 setae.	Endopod two jointed and each joint carries three setae. Exopod two jointed and has three terminal setae.

Family : *Lichomolgidae*

Pseudanthessius maximus Thompson and Scott. (Fig. 60).

Occurrence: Several females with eggs were taken in the month of March and kept in small vessels with fresh sea water. The nauplii were sketched soon after liberation.

Size: Length 0·45 m.m.

Colour and Description: The nauplius is colourless and it is longer than broad and is more or less egg shaped. The nauplius eye is present. The labrum is prominent and is broader towards the posterior end and narrow at the anterior end. There is a dark red spot towards the left side on the labrum. The posterior region is armed with two simple setae. Considerable amount of yolk is present inside the body and probably the nauplii do not feed at all.

Appendages: The *antennule* is three jointed, the terminal joint with three unequal setae. (Fig. 61). The *antenna* is biramous. The exopodite is longer than the endopodite. The exopodite is indistinctly two jointed, the terminal joint being armed with one lateral and three terminal setae. (Fig 62).

Mandible: Exopodite two jointed and carries two terminal setae. Endopodite is in two joints of which the second one has four setae and the first one, three setae. (Fig. 63).

Family: *Corycaeidae*.

CORYCAEUS SP.

Occurrence: The copepodites are present in the plankton in July, September and March. They are however, very common in March. The larva has the shape of the adult and its immaturity can be determined by the smaller size, the feathered nature of antenna and the three pairs of swimming feet.

Copepodite Stage II. (Fig. 64).

Size: Length 0·8 m.m.

Colour: The larva is colourless and transparent. The 'optic cups' are coloured deep blue.

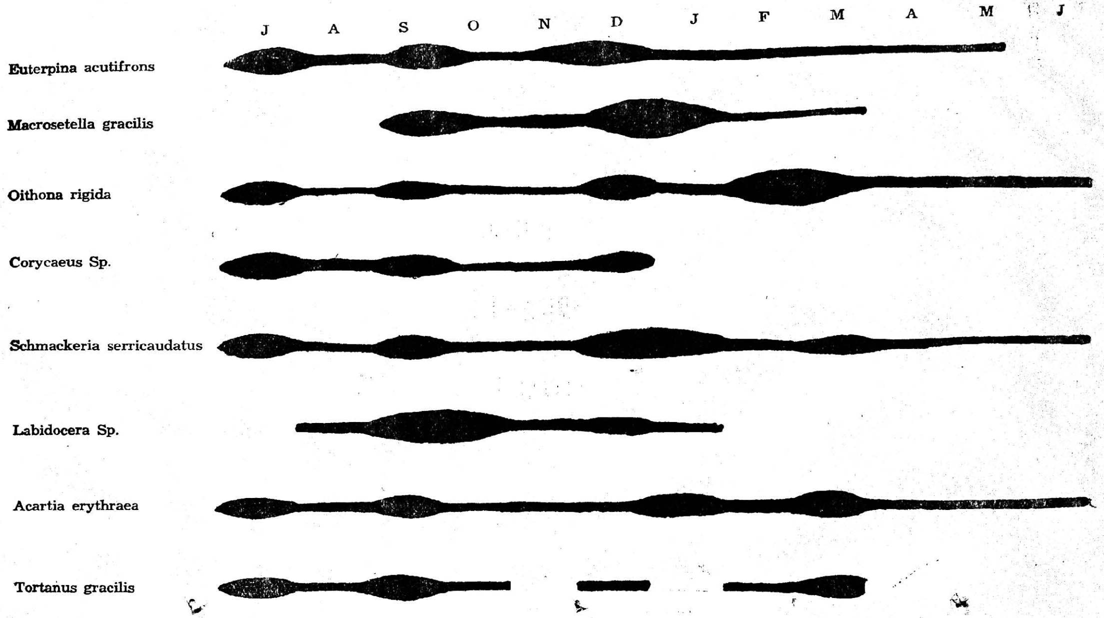
Body: The larva resembles the adult in general shape. The corneal lenses are present at the anterior end of the cephalosome. The urosome is indistinctly two jointed, the second joint being long and narrow. Caudal furca longer than broad and carries two apical long setae and two sub-apical short spines.

Appendages: The *antennule* is five jointed as in the adult. (Fig. 65). The *antenna* is of the usual shape as in the adult. The first joint has two long setae which are feathered. The second joint is very stout and is armed with a small tooth on the inner side. The third joint is very short and carries two short spines and two curved claws. (Fig. 66). Mouth parts as in the adult. *The first swimming feet*: biramous with a two jointed exopodite and endopodite. The first joint of the exopodite carries a small outer, serrated spine and the second joint three outer spines, one long, serrate terminal spine and four inner setae. The endopodite is shorter than the exopodite. The second endopod joint has four setae, three terminal and one sub-terminal (Fig. 67). *The second swimming feet* resemble the first one, with two jointed ramii. *Third swimming feet*: biramous with an unsegmented exopod and endopod. The exopodite is broad and has a short outer spine, a long apical spine and three inner setae. The endopodite is slightly smaller than the exopodite and has five setae. (Fig. 68). *Fourth swimming feet*: rudimentary

Remarks: Since a number of Corycaeus species are present in the Madras plankton, it is not possible to identify the present larva specifically.

TABLE I

Histogram showing the seasonal fluctuation of the larval stages of the Copepods in the Madras Plankton.



Remarks

The occurrence of the larvae in large numbers on certain days and their being very scarce on other days may be due to distribution of these larvae in patches in the sea as noted by Herdman (1923). This however, can be proved only when a continuous plankton recorder is used. Further, during March, July, September and December, when plankton was obtained roughly from the same area, on an average of eight days per month, the increase in numbers was sustained. Such a sustained abundance, cannot be explained as due to occurrence in patches. The abundance in March, repeated in July, September, and January for certain definite forms like *Oithona* and *Euterpina* has to be accounted for. In the same way, the total absence of some forms like *Labidocera, Macrosetella* and *Corycaeus* for example during April, May and June calls for explanation. (vide Table I).

The abundance of food due to diatom outbursts may be responsible for the abundance of larvae in July, September, December and March. But the larvae are not common when diatoms reach their maximum concentration in April, May and June. Observations made here agree with those as Clarke and Zinn (p. 486, 1937) who stated that "the spawning periods of *Calanus* did not occur at the times of diatom maxima". The increase in surface temperature and salinity of the water may explain the total absence of certain species in certain months. Besides all these physico-chemical explanations, it must be admitted, that seasonal intensity as well as cessation of gonadial activity is probable.

Acknowledgment

The author takes this opportunity to express his grateful thanks to Dr. C. P. Gnanamuthu, M.A., D.Sc., F.Z.S., Director, Zoology Laboratory, Madras University for guidance and help.

REFERENCES

Brian, A.* 1912. "I. Copepodii Harpacticoidi del Galfodi Genova" Strudi del Laboratoria Marino di Quarte de Mille presso Genova.

Chappuis, F. A.* 1916. Die metamorphose einiger Harpacticiden genera. Zool. Anz. Vol. 48, pp. 20-31.

Clarke, G. L. and Zinn, D, 1937. Seasonal production of zooplankton off Woodshole with special reference to *C. finmarchicus*. Biol. Bull. Vol. LXXIII, Vol. 31, 464-487.

Claus, C. 1858. Zur Anatomie und Entwicklungsgeschichite der Copepoden. Archiv. fur Naturgesh. Jahrb. 24, Band. I.

Dietritch, W. 1915. Die metamorphose der freilebenden Susswasser Copepoden. Zeit. wiss. Zool. Band 113. pp. 252-324.

Fraser, J. H. 1935-36. The occurrence, ecology and life history of *Tigriopus fulvus*. Journ. Marine Biol. Assn. (N.S.) Vol. 20, pp. 523-36.

Gibbons, S. G. and Ogilvie, H. S. 1933. Developmental stages of *O. helgolandica* and *O. spinirostris* with a note on the occurrence of body spines in Cyclopoid nauplii. Jour. Mar. Biol. Assn. (N. S.) Vol. 18, p. 529.

Grandori, R.* 1912. Strodi sullo sviluppo larvale dei copepodi pelagici. Redria. Firenze, Vol. VIII. pp. 360-456.

Gurney, R. 1930. Larval stages of Longipedia. Ibid. Vol. 16, pp. 461-474.

Gurney, R. 1931-32. British Fresh water Copepoda. Ray Society publications. 3 vols.

Gurney, R. 1933. Notes on some Copepods from Plymouth. Jour. Mar. Biol. Assn. (N. S.), Vol. 19, pp. 299-304.

Herdman, W. A. 1917. Spolia Runiana — II The distribution of some Diatoms and Copepoda throughout the year in the Irish sea. Jour. Linn. Soc. (Zoology) Vol. 34, pp. 95-126. (1918-22).

Johnson, M. W. 1934. The life history of the Copepod *Tortanus discaudatus*. Biol. Bull. Vol. 67, pp. 182.

Johnson, M. W. 1934. The developmental stages of the Copepod *Epilabidocera amphitrites* McMurrich. Biol. Bull. Vol. 67, p. 466.

Johnson, M. W. and Obson, J. B. 1948. The life history and biology of a marine Harpacticoid copepod, *Tisbe furcata** (Baird). Biol. Bull. Vol. 95, pp. 320-332.

Krishnaswamy, S. 1948. Stages in the development of a Harpacticoid Copepod *Macrosetella gracilis* (Dana). Curr. Sci. Vol. 18, 1948. (Abstract).

Krshnaswamy, S. 1950. Breeding seasons of the copepods in the Madras plankton. Proc. Ind. Sci. Cong. 37th session. (Abstract).

Lebour, M. V. 1916-1918. Stages in the life history of *Calanus finmarchicus* reared by Mr. L. R. Crawshay in Plymouth Laboratory. Jour. Mar. Biol. Assn. (N. S.), Vol. XI, pp. 1-17.

Menon, K. S. 1931. Preliminary account of the Madras Plankton. Rec. Ind. Mus. Vol. 33, Pt. IV, pp. 489-516.

Murphy, H. E. 1923. The life cycle of *Oithona nana* reared experimentally. Univ. Calif. Publ. in Zoology. Vol. 22, pp. 449-454.

Nicholls, A. G. 1934. The developmental stages of *Euchaeta norwegica* Boeck. Proc. Roy. Soc. Edin. Vol. LIV, pp. 31-50.

Nicholls, A. G. 1934. The larval stages of *Longipedia coronata* Claus, *L. scotti* Sars and *L. minor* Scott, T and A., with a description of the male of *L. scotti*. Jour. Mar. Biol. Assn. (N. S.) Vol. 20, pp. 29-45.

Nicholls, A. G. 1941. The developmental stages of *Metis jousseaummei* Richard. Ann. Mag. Nat. Hist. Vol. 7, pp. 317-328.

Oberg, M.* 1906. Die metamorphose der plankton — copepoden der Kieler Biocht.

Wis. Meerus urtersuch. d. deutschen Mere, in Kiel. N. F. Abt. Kiel. Vol. IX, pp. 37-103.

Sewell, R. B. S. 1929-32. The Copepods of the Indian Seas. Mem. Ind. Mus. Vol. X, Pt. I and II.

Wilson, C. B. 1932. Copepods of Woods Hole region. Bull. U. S. Nat. Mus. No. 158.

* Not referred to in original.

EXPLANATION OF TEXT-FIGURES

1. Centropages furcatus first Copepodite, dorsal view. (x 80).
2. " " " " First swimming feet. (x 400).
3. " " " " Second swimming feet.
4. " " " " Third swimming feet.
5. Centropages tenuiremis first Copepodite, darsal view (x 160),
6. " " " " First swimming feet. (x 400).
7. " " " " Terminal spine of the swimming feet. (x 600).
8. " " " " Antenna. (x 400).
9. " " " " Mandible Palp.
10. " " " " First Maxilla.
11. " " " " Second Maxilla.
12. " " " " Maxilliped.
13. Pseudodiaptomous aurivilli First Naupliar Stage. Dorsal view. (x 280).
14. " " " " Antennule. (x 600).
15. " " " " Antenna.
16. Schmackeria annandalei Nauplius Third Stage ? (x 400).
17. " " First Copepodite (x 120).
18. " " " " Antennule. (x 280).
19. " " " " First Swimming feet.
20. " serricaudatus, First Copepodite (x 120).
21. " " " " First Swimming Feet. (x 280).
22. " " " " Second Swimming Feet. (x 280).
23. Temora Sp. First Copepodite. (x 36).
24. " " " Antenna. (x 280).
25. " " " Mandible.
26. " " " First Maxilla.
27. " " " Second Maxilla.
28. " " " Maxilliped.
29. " " " First Swimming Feet.
30. Labidocera Sp. Nauplius, Second Stage ? (x 200).
31. Acartia erythraea First Copepodite Stage. (x 80).
32. " " " " First Swimming Feet. (x 280).
33. Tortanus gracilis Nauplius Third Stage ? (x 200).
34. " " First Copepodite, First Swimming Feet. (x 280).
35. Longipedia Sp. First Copepodite Stage. (x 120).
36. " " " " Antennule. (x 400).
37. " " " " First Swimming Feet. (x 400).
38. " " " " Second Swimming Feet. (x 400).
39. " " " " Third Swimming Feet. (x 400).
40. " Second Copepodite Stage Antennule. (x 400).
41. " " " " First Swimming Feet.
42. " " " " Second Swimming Feet.
43. " " " " Third Swimming Feet.
44. " " " " Fourth Swimming Feet.
45. Euterpina acutifrons Sixth Naupliar Stage ? (x 120).
46. " " First Copepodite. (x 80).
47. " " " " First Swimming Feet. (x 280).
48. " " " " Second Swimming Feet.

49. Macrosetella gracilis Second Nauplius Stage.
50. „ „ First Copepodite.
51. „ „ Antennule. (x 280).
52. „ „ First Swimming Feet.
53. Miracia efferata Second Copepodite ? (x 80).
54. „ „ „ „ First Swimming Feet. (x 280).
55. „ „ „ „ Second Swimming Feet.
56. „ „ „ „ Third Swimming Feet.
57. „ „ „ „ Fourth Swimming Feet. (x 280).
58. Oithona rigida, First Naupliar Stage. (x 400).
59. „ „ Mandible. (x 600).
60. Pseudanthessius maximus Nauplius, First Stage. (x 400).
61. „ „ „ Antennule. (x 180).
62. „ „ „ Antenna.
63. „ „ „ Mandible.
64. Corycaeus Sp. Second Copepodite ? (x 80).
65. „ „ Antennule. (x 280).
66. „ „ Antenna.
67 „ „ First Swimming Feet.
68. „ „ Third Swimming Feet.

FOOD FACTORS AND MIGRATION OF A FEW FISHES OF THE MADRAS COAST

BY

P. VIJIARAGHAVAN, B.SC.

Zoology Laboratory, Madras University

1. INTRODUCTION

Though the food and feeding habits of many Indian fishes have been studied by several, the relation of the food to the migratory habits of fishes, has not received the attention it deserves. Hornell and Ramaswamy Nayudu (1923), Job (1940), Chidambaram (1944), Venkataraman (1944), etc. have made brief comments, but the main body of the problem still remains obscure. An analysis of the stomach contents of some common fishes caught on the Madras Coast and a study of the increase and decrease of the quantity of the different items of diet, would throw light on the factors behind their migrations into and out of the area and their abundance and scarcity during the different months of the year. Such data would aid scientific fisheries.

2. MATERIAL AND METHODS

In the present paper, the results of two years study of the stomach contents of 1132 fishes belonging to clupeidae, Carangidae and Trichiuridae are presented. Analysis of the food of each of ten species, during the different months of the year, was made in the light of facts regarding the occurrence and fluctuations of the food items in the environment * and correlated with statistical data of the abundance and scarcity of the fishes. The data thus gathered are presented together with tentative conclusions regarding the factors affecting the migrations of these fishes into and out of the Madras shore area.

Of the total of 1132 fishes examined, 127 were carangids (*Gazza minuta*), 266 belonged to three species of *Trichiurus* and 739 clupeids belonging to 2 species of *Dussumieria* and 4 species of *Pellona*.

* The author is indebted to Mr. S. Krishnaswamy of the Department for information regarding planktonic organisms forming the food of fishes.

The observations pertaining to the typical genera of the families have been consolidated and presented in Tables I, II, III and IV. It is hoped that in a fuller paper, the data and conclusion for each of the ten species will be presented separately.

A classified list of species and genera of the three families studied is given below.

Family Carangidae, Genus Gazza (Rupp), Species *Gazza minuta* (Bloch).

Family Trichiuridae, Genus Trichiurus (Linn), Species *Trichiurus muticus* (Gray), *Trichiurus haumela* (Forak), *Trichiurus savala* (cuv and val).

Family Clupeidae, Genus Pallona (Cuv and val), Species *Pellona elongata* (Bennett), *Pellona indica* (Swains), *Pellona hoevenii* (Bleeker), *Pellona brachysoma* (Bleeker). Genus Dussumieria (Cuv and Val), Species *Dussumieria hasseltii* (Bleeker), *Dussumieria acuta* (Cuv and Val).

Table No. I — MONTHLY PERCENTAGES OF THE FOOD COMPONENTS IN PELLONA (CUV AND VAL)

Food Items	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Apl.	June	Average for one year
Teleosteans	9·01	1·00	4·02	12·11	9·77	8·14	1·49	7·29	–	20·0	7·28
Macrura	1·61	0·94	1·06	1·54	0·15	1·64	1·03	0·14	1·12	0·4	0·96
Anomura	0·24	–	0·36	0·12	0·03	–	–	–	–	–	0·10
Brachyura	0·96	0·41	0·64	0·56	1·06	0·32	2·51	0·42	–	–	0·69
Mysis	0·29	–	3·1	10·16	0·01	–	–	–	0·09	0·6	1·37
Entomostraca	0·33	0·42	1·34	0·19	2·72	0·21	0·80	0·37	0·56	0·15	0·71
Stomoatopoda	0·84	0·01	0·46	1·26	1·59	0·05	1·04	–	1·18	–	0·64
Miscellaneous Crustacea	0·05	–	0·02	0·04	0·11	–	0·02	0·01	–	–	0·02
Crustacean remains	2·95	3·34	7·86	5·18	3·15	8·51	2·21	1·5	3·68	2·43	4·18
Mollusca	0·16	0·03	0·11	0·06	0·11	0·13	0·06	0·11	0·01	0·02	0·08
Polychaets	0·11	–	2·05	0·98	0·25	0·06	–	–	–	–	0·35
Miscellaneous animal matter	0·01	0·01	0·02	0·02	0·01	0·01	0·03	0·03	0·09	–	0·02
Vegetable matter	–	0·03	0·06	0·02	0·13	0·04	–	–	0·08	0·72	0·11

Table No. II.—**MONTHLY PERCENTAGES IN THE FOOD COMPONENTS OF DUSSUMIERIA (CUV AND VAL)**

Food Items	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.	Average for 10 months	
Teleosteans	–	–	–	–	–	–	0·02	1·07	1·33	2·44	0·68	
Copepoda	11·02	38·99	13·8	0·48	19·11	7·84	4·42	5·63	0·5	·85	9·22	Crustaceans 26·13
Macrura	6·41	2·6	3·85	39·18	2·47	1·56	0·83	0·08	0·54	–	5·75	
Brachyura	0·24	1·75	2·36	1·47	2·85	24·48	4·16	1·28	0·08	4·41	4·71	
Anomura	2·78	–	0·59	0·57	1·11	3·0	0·36	0·05	–	0·04	0·85	
Mysis	5·62	1·59	3·67	–	2·49	–	0·03	5·04	–	–	1·84	
Other Entomostraca	0·52	0·07	0·37	–	–	0·05	–	–	–	–	0·09	
Stomoatopoda	–	4·7	0·84	–	–	–	4·73	0·16	2·27	0·81	1·99	
Crustacean remains	0·7	0·92	0·8	1·56	2·64	1·85	3·83	1·61	1·25	–	1·52	
Miscellaneous Crustaceans	–	0·05	0·04	0·04	0·31	0·37	–	0·78	0·02	–	0·16	
Mollusca	0·35	–	0·16	0·24	0·1	0·43	0·1	1·9	–	–	0·33	
Vegetable matter	0·22	3·27	1·17	0·46	0·33	0·55	0·17	2·2	0·5	0·15	0·95	
Chaetognatha	1·89	–	–	–	2·62	1·52	–	2·48	0·08	–	0·86	
Miscellaneous animal matter	0·17	0·03	0·77	0·06	0·19	0·2	0·24	0·67	0·13	0·04	0·25	

Table No. III.—THE MONTHLY PERCENTAGES OF THE FOOD COMPONENTS IN TRICHIURIDAE

Food Items	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Ap	9 months Average for	
Teleosteans	40·06	25·6	26·92	16·1	12·68	14·6	29·2	–	16·4	8·26	21·09	
Macrura	5·8	3·94	1·9	1·2	3·48	4·0	0·2	–	–	–	2·33	Crustaceans 5·27
Anomura	–	0·26	0·04	0·06	–	–	–	–	–	–	0·4	
Brachyura	0·02	1·64	1·8	0·1	0·04	–	–	–	0·92	0·14	0·52	
Copepoda	0·54	0·1	0·26	0·16	0·22	0·3	0·44	–	–	–	0·23	
Mysis	1·62	–	0·08	0·12	–	–	–	–	–	–	0·2	
Crustacean remains	1·18	1·1	–	0·28	6·2	–	–	–	–	2·06	1·2	
Stomoatopoda	–	0·6	0·32	0·52	–	–	–	–	–	–	0·16	
Miscellaneous Crustaceans	0·4	1·08	0·32	0·14	0·06	–	–	–	–	–	0·23	
Polychaets	0·04	0·04	0·56	–	–	–	–	–	–	–	0·07	
Mollusca	1·96	0·08	1·86	0·04	0·4	0·06	–	–	0·06	–	0·49	
Vegetable matter	–	1·32	2·24	0·06	0·42	0·52	1·2	–	–	–	0·64	

Table No. IV.—**MONTHLY PERCENTAGES IN THE FOOD COMPONENTS IN GENUS GAZZA REPRESENTED BY THE SINGLE SPECIES *GAZZA MINUTA* (BLOCH) THAT WAS STUDIED**

Food Items	July	Aug.	Sep.	Oct.	Nov.	Jan.	Feb.	Mar.	Apl.	
Penaeus	–	–	4·67	1·18	–	–	–	3·07	–	
Acetes	–	–	–	–	–	–	–	–	14·15	Crustaceans
Lucifer	1·82	–	1·67	0·01	6·29	0·14	–	1·81	–	Crustaceans
Other Sergestids	–	–	–	0·57	–	–	–	0·19	–	Crustaceans
Brachyuran remains	1·81	–	–	–	–	–	–	–	–	Crustaceans
Zoaea Brachyura	1·82	0·86	3·6	3·62	2·0	1·53	–	5·33	–	Crustaceans
Megalopa Brachyura	–	0·57	2·4	–	1·57	–	–	1·65	–	Crustaceans
Mysis	–	–	2·53	–	–	7·71	–	–	–	Crustaceans
Amphipods	0·18	0·18	–	–	–	–	–	0·03	–	Crustaceans
Pagurids	–	–	–	–	–	–	–	0·06	–	Crustaceans
Copepoda	2·18	–	2·8	8·23	0·28	–	4·5	3·38	8·13	Crustaceans
Crustacean remains	1·81	–	–	–	–	–	–	–	–	
Foraminifera	5·36	7·29	6·86	2·57	5·71	7·14	–	3·84	4·13	
Mollusca	1·0	6·85	5·4	2·29	4·42	9·14	1·0	6·35	2·25	
Polychaets	–	–	0·6	0·07	0·07	–	–	1·78	–	
Echinoderm	–	–	0·4	–	–	0·29	–	0·29	–	
Miscellaneous animal matter	–	–	–	0·3	–	–	–	–	2·25	
Vegetable matter	1·81	0·7	2·4	4·15	1·43	1·0	2·0	2·87	2·25	
Sand Particles	0·73	9·43	2·27	1·73	3·43	5·57	0·5	2·48	7·0	

3. Results of Observations

As observed by Devanesen and Chidambaram (1948), the four species of *Pellona* were seen (Vide Table I) to feed on young teleosteans and also on crustaceans like the macrurans, anomurans and the brachurans. The percentage of teleosteans consumed was greater compared to the other items of food, reaching a maximum of 20% in June. The occurrence of entomostracans and other smaller crustacea in the stomach contents show that the fish feeds by filtration when the larger prey are absent in the surroundings.

During the months, July to April the diet of the two species of *Dussumieria* was observed (Vide Table II) to change from small to larger crustacea. They were seen to feed chiefly on entomostracans and other larvel crustacea from July to January. But after January the predominant items were mysids, acetes, young prawns, post-larval teleosteans etc., and by April the diet was entirely of these with only a sprinkling of the smaller crustacea. This change in the diet of the species which feeds by filtration corresponds very closely with the change in the occurrence of these food organisms in the plankton. The final result of such a change in the feeding of the fish is that the dense shoals that were feeding on the plankton break up when later they are forced to hunt for their larger and more active prey. This might be a probable reason for the gradual decline in the amount of fish landed, for by March they become almost very rare : Bullen (1912) while investigating the feeding habits of Mackerel in the English Channel arrived at similar conclusions. He remarks, "During April, May and June the fish were feeding extensively on plankton and the nature of food gradually changed from being largely vegetable to almost wholly animal. It was not until the middle of June, that food of a larger character, but still wholly of the latter type, began to appear in stomach contents, and it is a well known fact that at about this time every year the drift fishery in the area under consideration begins to decline and hand lining commences. This condition is due to the fact that the dense shoals break up and the system of nutrition, which has hither to been one of filtration gives place to one of, what we may term 'selective feeding'".

The clupeids studied were plankton feeders deriving food from the plankton and are therefore more suited for a wider distribution. When the season begins, that is in July, August and also September, they are young and immature and were observed to be very active feeders. The reason for this shoreward migration is to feed

upon the immense quantities of zoo-and phyto-plankton that develops as the unicellular plants and animals multiply rapidly due to the north east monsoon. The peak occurrence of clupeids during July, August, September, October and November coinsides with the abundance of Trichiurus. By February the clupeids which arrived seven months before, ripen their gonads and leave the coastal waters. This off-shore migration can be explained as a spawning migration. Davenesen and Chidambaram suggest the period between March and September as the probable spawning season for species of *Dussumieria* of Pamban area. However, further information may be necessary to confirm such a conclusion.

The occurrence of clupeids like *Dussumieria* from July to April and of *Pellona* from July to February appears to attract the species of *Trichiurus* to the Madras coast. The abundance of such food renders the coast a feeding ground for their young and adolescent stages between July and December. As Hora (1944) says, " several important fisheries depend upon the relative abundance and scarcity of food and the annual and periodic fluctuations in their yields are mainly influenced by the amount of food available in any particular year."

The Trichiurids (*T. haumela and T. savala* being the common forms occurring in Madras) can be said to be inhabitants of the neritic zones, subsisting chiefly on the drift feeding teleosteans. These fast swimming fishes are eminently suited for chasing their prey. It is not clear on what evidence Venkataraman (1944) concluded that these are plankton feeders. An analysis (Vide Table III) shows that of the 27·6% of undigested matter, only 6·6% of the food is of planktonic organisms and even in their young stages these fish depend mainly on piscine fauna to the extent of 52·55% of their food. The clupeids form most of the food of these fish while the carangids rank second and it is interesting to note that the season for the ribbon-fish fishery commences in July when postlarval and young clupeids are abundant in the coastal waters of Madras. This is likely to affect the future clupeid fishery. But this loss in the fish income is more than compensated by the promotion of the fishery of Trichiurus which rank second in the economic grading of Madras fishes (Vide Moses 1922). When the season for these reaches its close by the end of January, a gradual fall in the amount of clupeids landed was observed. The presence of carangids and other teleosteans in the food contents of *Trichiurus* might be explained as having been taken in the absence of their favourite food, namely the clupeids, in the surroundings. The

carangids make their appearance while the *Trichiurus* are about to migrate from the coastal waters.

The months of February, March and April witnessed the maximum quantity of carangids (*Gazza minuta*) landed. Most of the fish examined were immature and adolescent stages. Similar to the observation of Davenesan and Chidambaram the analysis of stomach contents revealed (Vide Table IV) that they were bottom as well as plankton feeders. The planktonic organisms that formed the food were mostly crustacea like lucifer spp. mysis, copepoda, acetes, brachyuran larvae etc; and the bottom forms on which the fish fed where species of Foraminifera, Mollusca, Polychaeta, Echinodermata etc. Appreciable quantities of sand were observed to have been swollowed along with other food. Thus, these fish, being capable of considerable degree of vertical feeding often browsing on the bottom, are less affected by the changes in the pelagic fauna. As information regarding the bottom fauna is poor no definite conclusion can be drawn about the movement of these fish.

Summary

1. The diet and feeding habits of Thichiurus, Clupeids and Carangids are briefly described with their probable bearing on the commercial fisheries.

2. The Clupeids come to Madras by July and are present till April. The movement of clupeids into Madras waters seems to be influenced by the abundance of planktonic organisms along the coast. The Carangids appear in February and are present in large numbers till April. Species of Trichiurus occur from July to March feeding on the clupeids and reach their maximum number when the clupeids abound.

3. The migration of Species of Trichiurus to Madras area appears correlated with that of the clupeids on which they feed.

4. The inshore movements of Carangids can be explained only when factors behind plankton and the bottom fauna are better understood.

Acknowledgment

My thanks are due to Dr. C. P. Gnanamuthu, Director, University Zoology Research Laboratory, for his guidance.

Bibliography

BULLEN, 1912. Some notes on the feeding habits of Mackerel and certain Clupeids in the English Channel. Jour. Mar. Bol. Assoc. Vol. III.

CHIDAMBARAM, 1944. Food of the Indian Mackerel (*Rastrelliger kanagurta*) or the West Coast of Madras Presidency, Curr. Sci. August 1944.

DEVANESAN AND CHIDAMBARAM, 1948. The Common Food Fishes of The Madras Presidency, Department of Industries and Commerce, Madras.

HORNELL AND RAMASWAMY NAYUDU, 1923. A contribution to the Life History of the Indian Sardine with notes on the plankton of the Malabar Coast, Madras Fish. Bull. XVII.

JOB, T. J., 1940. An investigation on the nutrition of Perches of the Madras Coast. Rec. Indian, Mus. 1940.

MOSES, S. T., 1922. A statistical account of the fish supply of Madras. Madras Fish Bull.

VENKATARAMAN, R. S. 1944. The food of Ribbon fish. *Trichiurus* spp. Curr. sec. 1944.

MODULAR MULTIPLICATIVE FUNCTIONS *

BY

C. S. VENKATARAMAN, M.A., M.Sc.

1. *Introduction* †

There exist multiplicative functions [1] F(M, N) whose value is unaltered when N is increased by any multiple of M, that is to say, $F(M, N) = F(M, N + \lambda M)$ where λ is any integer. Such functions are called *Modular multiplicative functions* (or, briefly *Modular functions*) *with the modulus M.* A classical example of a Modular function is the well-known trignometric function $C_M(N)$ of Ramanujan, [2].

In a previous paper [3], the author investigated the properties of a class of multiplicatiye functions, known as the Ordinal functions and established an important relation between the Ordinal and the Modular functions. It is also shown [3] how some of the properties of the Modular functions can be easily deduced from the Ordinal functions. There are, however, certain properties, characteristic of the Modular functions, which cannot be derived from the Ordinal functions. Hence, it is the object of the present paper, to give an independent treatment of the Modular functions.

2. *Properties of the Modular functions*

Theorem (2·1). *If* F(M, N) *is a Modular function with the modulus* M, *then* F(M, N) *is multiplicative in* M *alone and* $F(M, N) = F(M, g)$ *where g is the greatest common divisor of* M, N.

To prove the first part, we require the

Lemma [2]. *The necessary and sufficient condition that a multiplicative function* F(M, N) *of the two arguments* M, N *should be multiplicative in one of the arguments (say)* M *is* $D_M(F) = E(N)$.

Now $F(1, 1) = 1$ since F(M, N) is multiplicative in M, N.

and $F(1, 1) = F(1, 1 + \overline{N-1}\cdot 1)$, since F(M, N) has the modulus M, taking $\lambda = N - 1$.

$= F(1, N)$.

Hence $F(1, N) = 1$

$$\text{(i.e.) } D_M(F) = E(N).$$

Therefore $F(M, N)$ is multiplicative in M alone.

To prove the second part, we note that as $F(M, N)$ is multiplicative in M, N,

$$F(M, N) = \prod_{i=1}^{r} F(p_i^{\alpha_i}, p_i^{\beta_i})$$

where $M = \prod_{i=1}^{r} p_i^{\alpha_i}$; $N = \prod_{i=1}^{r} p_i^{\beta_i}$; $\alpha_i \geqslant 0$, $\beta_i \geqslant 0$, $i = 1, \ldots, r$.

Since $F(M, N)$ has the modulus M, for any prime p

$$F(p^\alpha, p^\beta) = F(p^\alpha, p^\alpha) \text{ if } \beta > \alpha$$

For, $F(p^\alpha, p^\beta) = F(p^\alpha, p^\alpha + \overline{p^{\beta-\alpha} - 1} \cdot p^\alpha)$

$$= F(p^\alpha, p^\alpha), \text{ taking } \lambda = p^{\beta-\alpha} - 1.$$

Hence $\prod_{i=1}^{r} F(p_i^{\alpha_i}, p_i^{\beta_i}) = \prod_{i=1}^{r} F(p_i^{\alpha_i}, p_i^{\delta_i})$ where δ_i = smaller of α_i, β_i.

Therefore $F(M, N) = F(M, g)$, since $g = \prod_{i=1}^{r} p_i^{\delta_i}$

Corollary: (2·1·2) *A necessary condition for a multiplicative function* $F(M, N)$ *to have the modulus* M *is that* $D_M(F) = E(N)$.

(2·1·2) *If a multiplicative function* $F(M, N)$ *has* M *or* N *as a modulus, (i.e.)* $F(M,N) = F(M, N + \lambda M) = F(M + \lambda N, N)$, *then it is a function of the g. c. d. of the arguments.*

For, $F(M, N) = F(M, g) = F(g, g)$.

(2·1·3) *A necessary and sufficient condition for a multiplicative function of the form* $f_1(M) f_2(N)$ *to be a modular function with the modulus* M *is that* $f_2(N) = E(N)$; *and therefore any multiplicative function* $\Psi(M)$ *of a single argument can be regarded as a modular function* $\Psi(M) E(N)$.

Converse of Theorem (2·1) As a converse of the above theorem we have the

Theorem (2·2) *If* F(M, N) *is multiplicative in* M *alone and* F(M, N) = F(M, g) *then* F(M, N) *is a multiplicative function of both the arguments and has the modulus* M.

Proof : Let M, N. M', and N' be integers such that (MN, M'N') = 1.
Then (M, M') = (M, N') = (N, M') = (N, N') = 1. .. (A)
Suppose (M, N) = g ; (M', N') = g'.

Then (M, NN') = g ; (M' NN') = g', by virtue of (A)

Now F(M, N) = F(M, g) = F(M, NN')

and F(M', N') = F(M', g') = F(M', NN')

Therefore F(M, N) F(M', N') = F(M,NN') F(M', NN')
= F(MM', NN'), since F(M,N) is multiplicative in M, by hypothesis.

Thus F(M, N) is multiplicative in both M and N.

That F(M, N) has the modulus M is obvious from the hypothesis F(M, N) = F(M, g) since (M, N + λM = (M, N).

(2·3) *Form of the generating series of a modular function* F(M, N) *with the modulus* M.

The generating series of F(M, N) to a prime base p is given by

$$F_p(x, y) = \sum_{m=o}^{\infty} \sum_{n=o}^{\infty} F(p^m, p^n) x^m y^n$$

$$= \sum_{m=o}^{\infty} x^m [F(p^m, 1) + F(p^m, p) y + \ldots\ldots + F(p^m, p^m) y^m + F(p^m, p^{m+1}) y^{m+1} + \ldots]$$

$$= \sum_{m=o}^{\infty} x^m [F(p^m, 1) + F(p^m, p) y + \ldots + F(p^m, p^m) y^{m/1-y}],$$

since F(p^m, p^n) = F(p^m, p^m) when $n > m$.

(2·4) If F(M, N) is a multiplicative function of M and N, satisfying *any one* of the following statements :

(a) F(M, N) has the modulus M ;
(b) F(M, N) = F(M, g) ;
(c) F(M, N) is multiplicative in M alone ;

then (a) implies (b) and (c) ; (b) implies (c) and (a) ; whereas (c) does not imply either (a) or (b).

By Th. (2·1) (a) *implies* (b) *and* (c).

Now (b) *implies* (c), because $F(1, N) = F(1, 1)$ since $(1, N) = 1$
$= 1$ since $F(M, N)$ is multiplicative in M, N.

$$\text{(i.e.) } D_M(F) = E(N).$$

Since $(M, N) = g = (M, N + \lambda M)$, $F(M, N + \lambda M) = F(M, N)$ and therefore (b) *implies* (a) *also*.

We will next show that (c) *does not imply* either (a) or (b).

Now the generating series of $F(M, N)$ to the base p is

$$F_p(x, y) = \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} F(p^m, p^n)\, x^m y^n$$

$$= \sum_{m=0}^{\infty} x^m [F(p^m, 1) + F(p^m, p)\, y + \ldots + F(p^m, p^m)\, y^m + F(p^m, p^{m+1})\, y^{m+1} + \ldots]$$

$$= [F(1, 1) + F(1, p)\, y + \ldots.] + \sum_{m=1}^{\infty} x^m [F(p^m, 1) + \ldots + F(p^m, p^m)\, y^m + F(p^m, p^{m+1})\, y^{m+1} + \ldots.]$$

$$= \frac{1}{1-y} + \sum_{m=1}^{\infty} x^m [F(p^m, 1) + \ldots + F(p^m, p^m)\, y^m + F(p^m, p^{m+1})\, y^{m+1} + \ldots]$$

since by assumption $F(M, N)$ has the property (c) and therefore $F(1, N) = 1$.

But the generating series of $F(M, N)$ if either (a) or (b) is to be true (of course each implies the other) must be of the form

$$F_p(x, y) = \frac{1}{1-y} + \sum_{m=1}^{\infty} [F(p^m, 1) + \ldots + F(p^m, p^m)\, y^m + F(p^m, p^m)\, y^{m+1} + \ldots],$$

by using (2·3) ;

(i.e.) the coefficients of y^m and all higher powers of y are equal.

Thus it is clear that (c) does not imply (a) or (b).

Relation between a Modular function and Euler's Φ function.

Theorem (2·5) *If* F(M, N) *is a Modular function with the modulus* M, *then* $\Sigma F(M, Nh) = g \sum_{\delta | M/g} F(M, g\delta)\ \Phi\ (M/g\delta)$, *where h runs through a complete set of residues* (*mod* M).

For the proof, we require the following

Lemma [4]. *If* $(a, m) = g$, $a = ga'$, $m = gm'$, *and* g/K, *the remainders of the series* $K, K + a, \ldots, K + (m-1)a$ *when divided by* m, *are the numbers* ${}^0g, {}^1g, {}^2g, \ldots, {}^{(m-1)}g$, *continually repeated in a certain order.*

Proof : Since F(M, N) is a modular function with the modulus M,

$$F(M, N) = F(M, N + \lambda M) \qquad \ldots \qquad \ldots \qquad (2 \cdot 5 \cdot 1)$$

$$\text{and } F(M, N) = F(M, g) \qquad \ldots \qquad \ldots \qquad (2 \cdot 5 \cdot 2)$$

From the lemma, by writing $m = M$, $a = K = N$ and using (2·5·1) and (2·5·2), we see that

$\Sigma F(M, Nh) = g \Sigma F(M, gr)$ where r runs through a complete set of residues (mod M/g).

Now $(M, gr) = g(M/g, r) = g\delta$ if $(M/g, r) = \delta$.

Therefore $F(M, gr) = F(M, g\delta)$.

Arguing as in Th. (4·1·2) of [2], it follows that

$$\Sigma F(M, gr) = \sum_{\delta | M/g} F(M, g\delta)\ \Phi(M/g\delta).$$

Hence $\Sigma F(M, Nh) = g\Sigma F(M, g\delta)\ \Phi\ (M/g\delta)$, where δ runs through all the divisors of M/g.

Corollary : If $N = 1$, $\sum_{h=1}^{M} F(M, h) = \sum_{\delta | M} F(M, \delta)\ \Phi(M/\delta) = f(M, M)$

where $f(M, N) = F(M, N).\ E_o(M)\Phi(N)$.

Theorem (2·6) *If* $F(M, N)$ *is a modular function with the modulus* M, *then* $\Sigma F(M, Nr) = \Phi(M) F(M, g)$, *where* r *runs through all integers not greater than and prime to* M.

Proof: For, if $(M, r) = 1$, then $(M, Nr) = (M, N) = g$. Since there are $\Phi(M)$ numbers not greater than and prime to M, we have by (2·5·2),

$$\Sigma F(M, Nr) = \Phi(M) F(M, g).$$

Corollary: $\Sigma F(M, Nr) = \dfrac{\Phi(M)}{\Phi(M/g)} \Sigma F(M, N\delta)$, *where* δ *runs through numbers not greater than and prime to* M/g.

For, $(M, N\delta) = g\left(\dfrac{M}{g}, \dfrac{N}{g}\delta\right) = g$ since $\left(\dfrac{N}{g}, \dfrac{M}{g}\right) = \left(\delta, \dfrac{M}{g}\right) = 1.$

As δ runs through the $\Phi\left(\dfrac{M}{g}\right)$ numbers not greater than and prime to M/g, $N\delta$ runs through the $\Phi\left(\dfrac{M}{g}\right)$ numbers not greater than M and having the $g\cdot c\cdot d$, g with M.

$$\text{Therefore } \Sigma F(M, N\delta) = \Phi\left(\frac{M}{g}\right) F(M, g) = \frac{\Phi(M/g)}{\Phi(M)} \Sigma F(M, Nr)$$

$$\text{(i.e.) } \Sigma F(M, Nr) = \frac{\Phi(M)}{\Phi(M/g)} \Sigma F(M, N\delta).$$

Product, composite and inverse of Modular functions.

Theorem (2·7). *The product of modular functions is a modular function; whereas the composite and inverse of modular functions are not modular functions.*

The first part follows readily from the definition of a modular function.

Regarding the composite let

$f(M, N) = F(M, N).\ \psi(M, N)$, where $F(M, N)$ and $\psi(M, N)$ are modular functions with the modulus M.

Then, $D_M(f) = D_M(F) . D_M(\psi) = E(N)$, using (2·1·1)

$= d(N)$, the number of divisors of N.

Therefore $D_M(f) \neq E(N)$ for all values of N and thus the necessary condition for modularity is not satisfied.

If $F^{-1}(M, N)$ is the inverse of $F(M. N)$,

$D_M(F^{-1}) = \{D_M(F)\}^{-1} = \{E(N)\}^{-1} = \mu(N)$; and hence the inverse is not a modular function.

Theorem (2·8) *If* $F(M, N)$ *and* $\psi(M, N)$ *are modular functions with the modulus M and* $f(M, N) = \sum_{d|M} F(\frac{M}{d} N)\ \psi(d, N)$ *then* $f(M, N)$ *is a modular function with the modulus* M.

Proof : First, we will show that $f(M, N)$ is multiplicative in M, N.

Let $(M'N', MN) = 1$.

Then $(M', M) = (M', N) = (N', M) = (N' N) = 1$ .. (2·8·1)

If $d|M$, $d'|M'$, then by (2·8·1) $(d', d) = (N'd', Nd) =$

$$\left(\frac{M'}{d'}, \frac{M}{d}\right) \leq \left(\frac{M'N'}{d'}\ \frac{MN}{d}\right) = 1 \quad .. \ (2·8·2)$$

Also $dd'|MM'$ and every divisor of MM' can be uniquely expressed in the form dd' where $d|M$ and $d'|M'$.

Hence, $f(M, N)\ f(M',N') = \{\sum_{d|M} F(\frac{M}{d},N)\ \psi(d, N)\}\{\sum_{d'M'} F(\frac{M'}{d}N')$
$\Psi(d', N')\}$

$$= \sum_{dd'|MM'} F(\frac{MM'}{dd'}, NN')\ \psi(dd'\ NN')$$

since F (N, N) and $\psi(M, N)$ are multiplicative and by using (2·8·2).

$= f(MM', NN')$.

Thus $f(M, N)$ is multiplicative in M, N.

$f(M, N)$ has the modulus M, because

$$f(M, N+\lambda M) = \Sigma\, F(\frac{M}{d}, N+\lambda M)\, \psi(d, N+\lambda M)$$

$$= \sum_{d|M} F\left(\frac{M}{d'}, N+\lambda d.\frac{M}{d}\right) \psi\left((d, N+\lambda\frac{M}{d}\, d)\right)$$

$$= \sum_{d|M} F\left(\frac{M}{d} N\right) \psi(d, N),$$ as $F(M, N)$ and $\psi(M, N)$ have the Modulus M

$$= f(M, N).$$

Hence $f(M, N)$ is a modular function with the modulus M.

Corollary: If $F(M, N)$ *is a modular function with the modulus* M, *then* $\sum_{d|M} F\left(\frac{M}{d}, N\right)$ *is also a modular function.*

This follows by taking $\psi(M, N) = E(M) E(N)$.

Theorem (2·9) *If* $F(M, N)$ *is a modular function with the Modulus* M *and* $\psi(M, N)$ *is any multiplicative function of two arguments, then* $f(M, N) = \sum_{d|g} F\left(\frac{M}{d}, \frac{N}{d}\right) \psi(d, d)$ *where* $g = (M, N)$, *is a modular function with* M *as a modulus.*

The multiplicative property of $f(M, N)$ in M, N can be established as in Th. (2·8) *or* it can be easily seen from the fact that $f(M, N)$ is the composite of $F(M, N)$ and the principal function-equivalent to $\psi(M, N)$.

Now $f(M, N)$ has the modulus M, for,

$$f(M, N+\lambda M) = \sum_{d|g} F\left(\frac{M}{d} \frac{N+\lambda M}{d}\right) \psi(d, d),$$ since $(M, N+\lambda M) = (M, N)$.

$$= \sum_{d|g} F(\frac{M}{d}, \frac{N}{d} + \lambda\frac{M}{d})\, \psi(d, d)$$

$= \sum_{d|g} F(\frac{M}{d}, \frac{N}{d})\, \psi(d, d)$ since $F(M, N)$ has the modulus M by hypothesis.

$= f(M, N)$.

Thus $f(M, N)$ is a modular function with the modulus M.

Corollary: $\Sigma F(\frac{M}{d}\ \frac{N}{d})$, *summed for the common divisors of* M, N *is a modular function with the modulus M.*

The nature of the Cardinal Component of a Modular function.

Theorem (2·10) *The cardinal component of a modular function* $F(M, N)$ *with the modulus* M, *is an Ordinal function with* M *as the major argument.*

Proof: For, if $C(M, N)$ is the cordinal component of $F(M, N)$, by 3, [2],

$$C(M, N) = F(M, N).\ \overset{-1}{F}(M, 1)\mu(N), \text{ since } \overset{-1}{F}(1, N) = \{E(N)\}^{-1}.$$

As $C(M, N)$ is multiplicative, it is sufficient if we find $C(p^m, p^n)$ for a prime p.

$$\text{Now } C(p^m, p^n) = F(p^m, p^n)\ \overset{-1}{F}(p^m, 1)\mu(p^n)$$

$$= \sum_{d/p^m} \sum_{\delta/p^n} F(\frac{p^m}{d}, \frac{p^n}{\delta})\ F\ (d, 1)\mu((\delta)$$

$$= \sum_{d|p^m} \overset{-1}{F}(d, 1) \sum_{\delta|p^n} F(\frac{p^m}{d}, \frac{p^n}{\delta})\mu(\delta)$$

$$= \sum_{d|p^m} \overset{-1}{F}(d, 1)\ \{F(\frac{p^m}{d}\ p^n) - F(\frac{p^m}{d}, h^{n-1})\}$$

Since $\mu(1) = 1$, $\mu(p) = -1$ and $\mu(p^k) = 0$ for $k > 1$.

$$= \{F(p^m, p^n) - F(p^m, p^{n-1}\}$$

$$+ \overset{-1}{F}(p, 1)\ \{F(p^{m-1}, p^n) - F(p^{m-1}, p^{n-1})\}$$

$$+ \overset{-1}{F}(p^2, 1) \{F(p^{m-2}\ p^n) - F(p^{m-2}, p^{n-1})\} + \ldots\ldots$$

$$+ \overset{-1}{F}(p^m, 1) \{F(1, p^n) - F(1, p^{n-1})\}.$$

Now if $n > m$, $n - 1 \geqslant m$; $p^n > p^m$ and $p^n \chi p^m$.

Therefore $F(p^m, p^n) = F(p^m, p^m)$; $F(p^m, p^{n-1}) = F(p^m, p^m)$

$F(p^{m-1}, p^n) = F(p^{m-1}, p^{m-1})$; $F(p^{m-1}, p^{n-1}) = F(p^{n-1}, p^{m-1}$
and so on,

(since $F(M, N)$ is a modular function).

Thus the terms on the right side vanish and therefore

$$C(p^m, p^n) = 0 \text{ if } p^n \chi p^m.$$

Hence $C(M, N) = 0$ if $N \chi M$.

(i.e.) $C(M, N)$ is an ordinal function with the major argument M.

References

1. R. Vaidyanathaswamy: The Theory of Multiplicative Arithmetic functions. *Trans. Am. Math. Soc.* Vol. 33, pp. 579-662, (1931).

2. C. S. Venkataraman: A New Identical Equation for Multiplicative functions of two arguments and its applications to Ramanujan's sum $C_M(N)$. *Proc. Ind. Acad. Sci.* 24A, (1946), pp. 518-29.

3. C. S. Venkataraman: The Ordinal correspondence and certain classes of multiplicative functions of two arguments. *Jour. Ind. Math. Soc.*, Vol. X, 81-101, (1946).

4. Chrystal: Algebra Pt. II, pp. 547-50.

* This paper is a slight development of a part of a thesis approved for the M.Sc. degree of the University of Madras and I am indebted to Dr. R. Vaidyanathaswamy for his guidance.

† The definitions and notations, unless otherwise explained, are the same as in the references, indicated by the numbers in [ ] and cited fully at the end of the paper.

SEED-BORNE FUNGI AND LOSS OF COTTON SEED VIABILITY *

BY

C. S. VENKATRAM, B.Sc. (HONS.), M.Sc.
University Botany Laboratory, Madras

INTRODUCTION

Loss of seed viability in storage consequential on fungal growth has been studied as an academic problem in many countries (Barre, 1912; Lehman, 1925; Ludwig, 1925; Shands, 1937; Duggar and Koehler, 1944; Tervet, 1945; Arndt, 1946 a, b). Factors contributing to loss of cotton seed viability in storage have been enumerated by Simpson (1935, 1942, 1946), Simpson and Stone (1935) and Simpson and Miller (1944). In this country, however, very few investigations into the causes of seed decay in storage have been undertaken in the past, Sonavne (1928, 1934) and Saran (1945). Cotton seeds from selfed flowers from the experimental plots of this laboratory as well as pure line seeds obtained from the Provincial Agricultural Department when stored under laboratory conditions from time to time showed progressive decrease in germination percentage as storage time increased. However, it should be stated that storage in sealed containers showed considerably less loss in viability than when stored in open bags of cloth and paper. The object of this work was not only to record the occurrence of pathogenic and saprophytic fungi both externally and internally to the cotton seed and what effect it had on loss in seed viability but also to determine the exact role played by these fungal isolates in the dissemination of pre-and post-emergence diseases of the growing cotton seedling under varying soil conditions. Many of the saprophytic fungi as well as those that are parasitic on cotton have been isolated from within and without the cotton seed by Barre (1912), Crawford (1923), Elliot (1923), Taubenhaus and Ezekiel (1932), Kulkarni (1934), Dastur (1934), Bain (1939), Ray and McLaughlin (1942) and Rudolph and Harrison (1945).

* Part of thesis in fulfilment of the Master of Science Degree, University of Madras.

It is, however, significant to note that considerable difference of opinion exists in literature on the occurrence of many species of *Fusarium* especially *Fusartum vasinfectum* within the cotton seed. It is now generally agreed that in the dissemination of pathogenic fungi through seed either external or internal, knowledge of their occurrence and distribution have a profound effect on physical and chemical methods employed in seed treatment. This work, therefore, aims at studying the relation of storage conditions to viability of cotton seeds with particular reference to role of various fungi in loss of seed viability when germinated under varying soil conditions in the presence of seed-borne isolates.

MATERIALS AND METHODS

Standard mycological techniques advocated by Rawlins (1933) were followed throughout the investigations.

pH determinations of media were made using Beckman's pH meter with a glass electrode. pH findings of soil samples were carried out according to methods described by Piper (1944).

Single spore cultures were made of all fungal isolates with the help of the 'dilution plate technique'. The agar disc method of inoculation as tried by Garrett (1936) for *Ophiobolus graminis* was used in all experiments wherein rates of fungal growth had to be compared. Saturation capacity of soil was determined by the Keen — Raczkowski method (Keen and Raczkowski, 1921).

For determination of internal moisture content of cotton seeds, these were dried at 100°C. for 24 hours or ot 130°C. for 2 hours in an electric oven, thereafter cooled in a desiccator and weighed, the process being repeated till a constant weight was obtained. Moisture percentages of seeds are reported on a wet weight basis. This method was adopted following the recommendations made by A.O.A.C. (1945).

Three varieties of cotton seeds were used in the studies on the effect of storage conditions on viability:

(i) Karunganni 1 (*Gossypium arboreum*).

(ii) Guntur 1 (*Gossypium arboreum*).

(iii) Hagari 1 (*Gossypium herbaceum*).

All seeds employed in these investigations were collected from self-pollinated plants and obtained from the Agricultural Depart-

ment, Madras. The three samples were divided into two lots each one being retained as normal fuzzy seeds, whereas the other lot was delinted with concentrated sulphuric acid following the method recommended by Brown (1933). The moisture adjustment of seed samples was carried out in accordance with the method followed by Simpson (1935) which is as follows:—The seed sample is first spread out evenly on large flat galvanized tray and the initial moisture content of the seeds in the sample determined. For increasing the moisture content of the seed sample, water is sprinkled over the seeds and the latter thoroughly mixed. Addition of water to the seed lot is continued until the required moisture level of the seed sample is obtained. For reducing the moisture content of seeds, the sample is dried in the sun or in an electric oven at 35°C. The different seed samples at the various moisture levels were stored in sealed specimen tubes and in glass bottles with tight fitting lids to prevent fluctuations in internal seed moisture content and this condition represented seeds kept under sealed storage. Those held in open storage were placed in tin containers without lids. Samplings of the seed lots for germination and infection tests were made at the beginning of the experiment and after suitable periods of incubation.

A modification of the 'Ulster' method given by Muskett and Malone (1941) was adopted in the studies on the cotton seed microflora; the modification being essentially in the direction of using a lower concentration of nutrient media. All seeds were surface sterilized with mercuric chloride (1:1000) for one and one half minutes with subsequent washing in four changes of sterile water prior to plating out on 1 per cent consolidated malt extract media in sterile 9 cm. Petri dishes and over agar slopes in culture tubes. Ten seeds were plated out in each of the 9 cm. Petri dishes and one seed over every agar slope in each of the culture tubes. All fuzzy seeds were delinted with sulphuric acid prior to surface sterilization and plating out, to obviate any vitiation from the microflora on the exterior of the seed.

The cultures of fungi isolated in these studies were provisionally identified in this laboratory and were later confirmed by the Mycology Department of the Agricultural Research Institute, New Delhi.

Two types of soils were used in the experiments to assess the pathogenicity of the various fungal isolates to cotton:

(i) Composted soil consisting of garden soil, sand and dung manure in the proportion of 8:1:1 with saturation capacity approximating 38 per cent.

(ii) Black cotton soil from Udamalpet cotton growing tracts (Coimbatore District, Madras Presidency) with saturation capacity of 68 per cent.

EXPERIMENTAL

A. Storage conditions and seed viability :—Role of fungi in loss of viability of cotton seed in storage and studies on the resultant effects of microbial activity and various storage conditions governing them on longevity of the seed.

Experiment I.

Fuzzy and acid delinted seeds of K. 1 variety were taken and portions of these two lots were adjusted to 6, 9, and 12 per cent moisture content. Samples of both the acid delinted and fuzzy lots at the three moisture levels were stored at 0°C., 10°C. and laboratory room temperature (28°-30°C.) in sealed specimen tubes. In addition the samples under the room temperature of storage were further divided into two portions, one being stored in open tin containers while the other portion remained stored in sealed specimen tubes. Sampling of the seed lots for germination and infection tests were made at the begining of the experiment and after 3, 6, 9, 12 and 15 months storage. In addition both fuzzy and acid delinted cotton seeds of G. 1 variety with internal moisture contents of 9, 13 and 16 per cent were kept under sealed storage conditions at 28°-30°C. Samplings for germination and infection tests were made after 2, 5, 9, 12, and 15 weeks' storage. 100 seeds from each of the various samples under different storage conditions were cultured over 2 per cent agar media in Petri dishes and culture tubes after preliminary surface sterilization as described earlier (vide 'Materials and Methods'). Observations on the germination and infection of the seeds were made up to 10 days after culturing. The term 'Viable seed' has been used to designate the percentage of seeds that formed normal radicles. Those that formed abnormal radicles were grouped separately under viable seeds and are not included in these results for the sake of brevity. A specimen table (1) is, however, presented showing the basis on which the results of this experiment were recorded. Some of the salient features of the experiment are incorporated in Tables I to III and Text Figs. I to VII, and the following conclusions derived from this experiment :

TABLE I — Showing the percentage germination and infection of delinted K. 1 seeds with 9 per cent moisture content after different periods of storage at 28° to 30°C. in open and sealed containers.

Storage in open containers.

Period of storage	Viable seeds. Producing normal seedlings		Viable seeds. Producing abnormal seedlings		Emergence in soil	Non-viable seeds			
	Total	Infected	Total	Infected		Total	Infected Total	Infected Fungi	Infected Bacteria
Initial	81	1	0	0	80	19	10	9	1
3 months	32	1	21	11	30	47	34	28	6
6 months	12	2	32	20	8	56	45	28	17
9 months	0	0	0	0	0	100	67	18	49
12 months	0	0*	0	0	0	100	78	15	63
15 months	0	0	0	0	0	100	99	13	86
Storage in sealed containers.									
Initial	81	1	0	0	80	19	10	9	1
3 months	54	2	19	9	52	27	24	18	6
6 months	43	2	13	10	40	44	28	20	8
9 months	30	2	13	7	24	57	18	8	10
12 months	21	0	11	4	18	68	35	4	31
15 months	4	0	9	0	2	87	50	0	50

* Represents infection by fungi.

TABLE II — Showing percentage germination and infection of K. 1 seeds with initial moisture content of 9 and 12 per cent after different periods of storage at 10° and 0°C.

Fuzzy

Period of storage	Seeds with 9 per cent moisture content.							
	Storage temperature 10°C.				Storage temperature 0°C.			
	Viable non-viable	Percentage infection of non-viable seeds			Viable non-viable:	Percentage infection of non-viable seeds		
		Total	Fungi	Bacteria		Total	Fungi	Bacteria
Initial	81 : 19	10	9	1	81 : 19	10	9	1
3 months	74 : 22	19	14	5	79 : 20	14	11	3
6 months	71 : 23	22	16	6	78 : 20	18	13	5
9 months	66 : 31	23	17	6	77 : 23	21	15	6
12 months	66 : 32	18	10	8	76 : 24	17	11	6
15 months	65 : 35	16	6	10	73 : 27	16	7	9
Acid-delinted seeds								
Initial	81 : 19	10	9	1	81 : 19	10	9	1
3 months	79 : 17	17	10	7	78 : 20	16	9	7
6 months	73 : 22	20	12	8	75 : 22	20	10	10
9 months	71 : 23	22	14	8	70 : 29	28	11	17
12 months	67 : 31	26	7	19	61 : 39	32	8	24
15 months	64 : 34	31	4	27	52 : 48	42	5	37

* Contained 5% seeds infected with both fungi and bacteria.

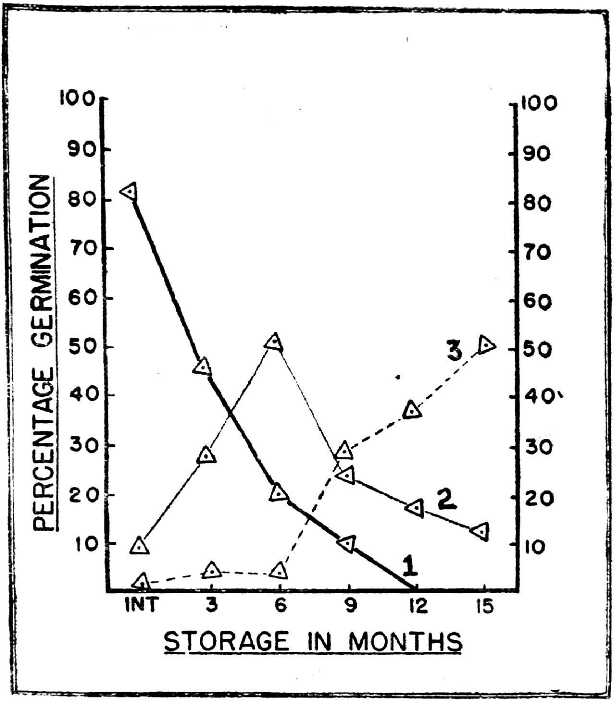
Period of storage	Seeds with 12 per cent moisture content							
	Storage temperature 10°C.				Storage temperature 0°C.			
	Viable : non-viable	Percentage infection of non-viable seeds			Viable : non-viable	Percentage infection of non-viable seeds		
		Total	Fungi	Bacteria		Total	Fungi	Bacteria
Initial	81 : 19	10	9	1	81 : 19	10	9	1
3 months	72 : 28	28	28*	5	77 : 22	20	16	4
6 months	70 : 30	30	30**	7	77 : 22	22	16	6
9 months	65 : 34	34	27	7	75 : 25	23	16	7
12 months	60 : 28	35	26	9	71 : 29	24	15	9
15 months	57 : 43	27	15	12	70 : 30	27	14	13
Acid-delinted seeds								
Initial	81 : 19	10	9	1	81 : 19	10	9	1
3 months	61 : 36	30	26	4	40 : 50	42	11	31
6 months	50 : 40	35	27	8	15 : 73	64	13	51
9 months	42 : 45	43	28	15	6 : 84	67	14	53
12 months	22 : 71	60	22	38	0 : 100	69	14	55
15 months	16 : 80	80	32	48	0 : 100	90	32	58

** Contained 7% seeds infected with both fungi and bacteria.

TABLE III — Showing percentage germination and infection of K. 1 seeds (Fuzzy) with 9 and 12 per cent. moisture content stored in open and sealed containers at laboratory room temperature (28° to 30° C.).

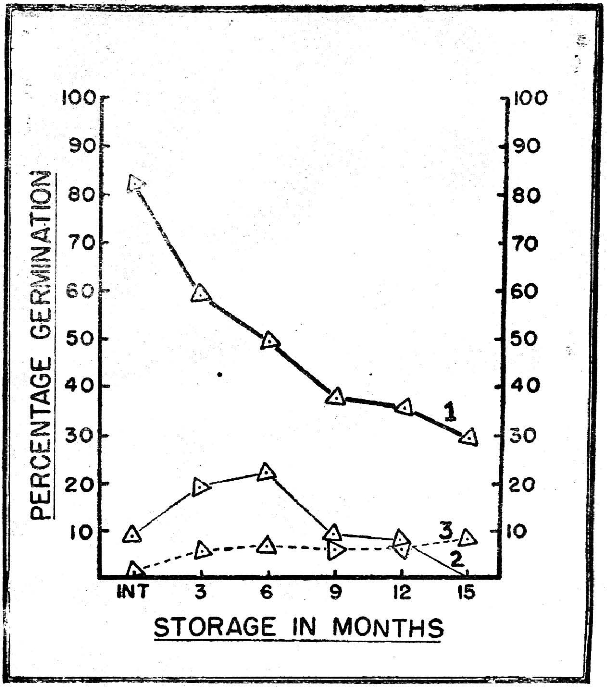
Open Containers.

Period of storage	Seeds with 9 per cent moisture content				Seeds with 12 per cent moisture content			
	Viable : non-viable seeds	Percentage infection of non-viable seeds			Viable : non-viable seeds	Percentage infection of non-viable seeds		
		Total	Fungi	Bacteria		Total	Fungi	Bacteria
Initial	81 : 19	10	9	1	81 : 19	10	9	1
3 months	36 : 52	41	37	4	19 : 77	77	72	5
6 months	20 : 61	58	48	10	10 : 89	89	78	11
9 months	0 : 100	70	42	28	0 : 100	84	49	35
12 months	0 : 100	65	26	39	0 : 100	71	27	44
15 months	0 : 100	74	16	58	0 : 100	92	18	74
Storage in sealed containers.								
Initial	81 : 19	10	9	1	81 : 19	10	9	1
3 months	57 : 31	29	25	4	0 : 96	92	86	6
6 months	30 : 55	49	43	6	0 : 100	100	100	0
9 months	23 : 67	28	18	10	0 : 100	65	14	51
12 months	16 : 77	27	8	19	0 : 100	72	10	62
15 months	3 : 92	40	0	40	0 : 100	92	0	92



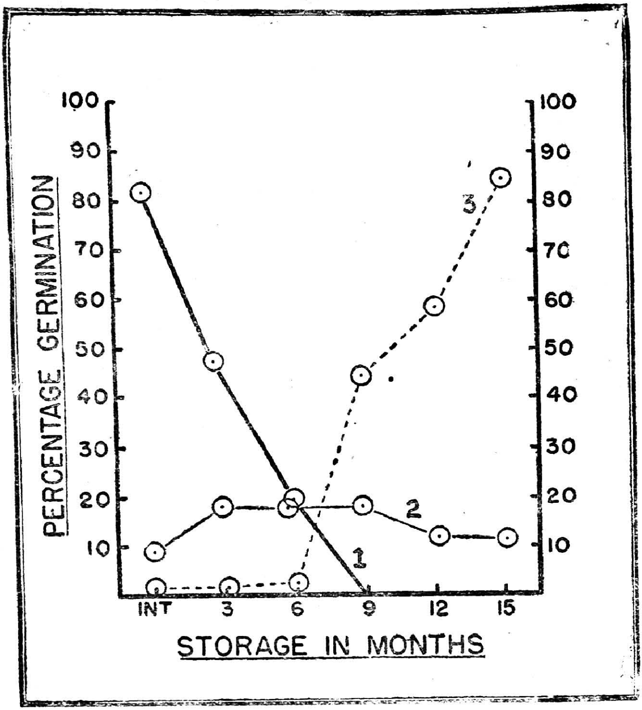
Text-fig. I. Showing the germination and infection of fuzzy seeds of K. 1 variety with 6 per cent. moisture content after different periods of Storage at 28°-30°C. in open containers.

Percentage germination—Curve 1.
Percentage infection by fungi—Curve 2.
Percentage infection by bacteria—Curve 3.



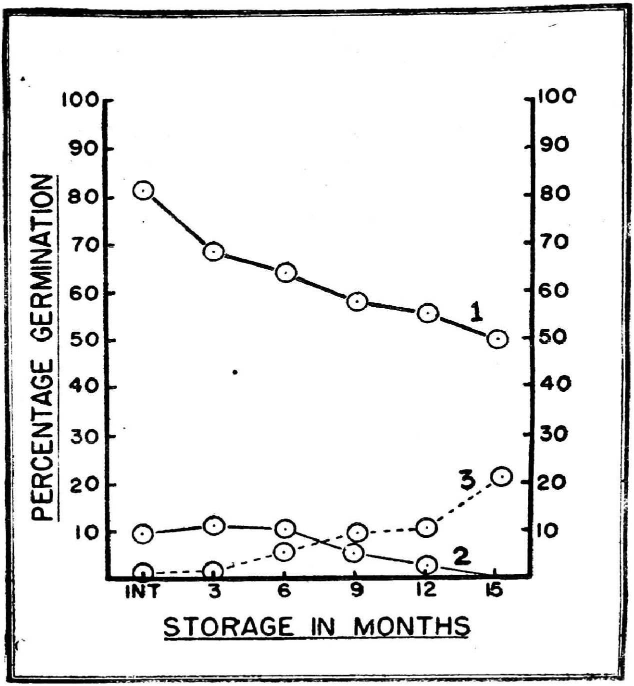
Text-fig. II. Showing the germination and infection of fuzzy seeds of K. 1 variety with 6 per cent. moisture content after different periods of storage at 28°-30° C. in sealed containers.

Percentage germination — Curve 1.
Percentage infection by fungi — Curve 2.
Percentage infection by bacteria — Curve 3.



Text-fig. III. Showing the germination and infection of delinted seeds of K. 1 variety with 6 per cent. moisture content after different periods of storage at 28°-30°C. in open containers.

Percentage germination — Curve 1.
Percentage infection by fungi — Curve 2.
Percentage infection by bacteria — Curve 3.

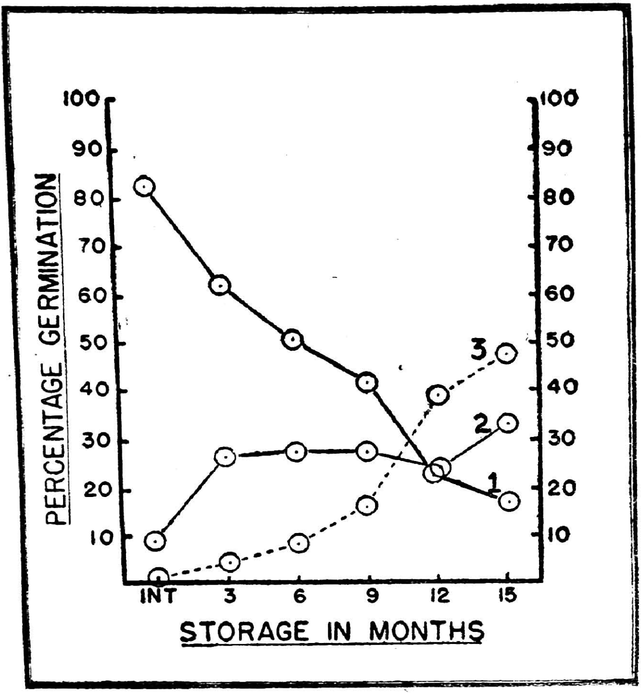


Text-fig. IV. Showing the germination of delinted seeds of K. 1 variety with 6 per cent. moisture content after storage for different periods at 28°-30°C. in sealed containers.

Percentage germination — Curve 1.

Percentage infection by fungi — Curve 2.

Percentage infection by bacteria — Curve 3.

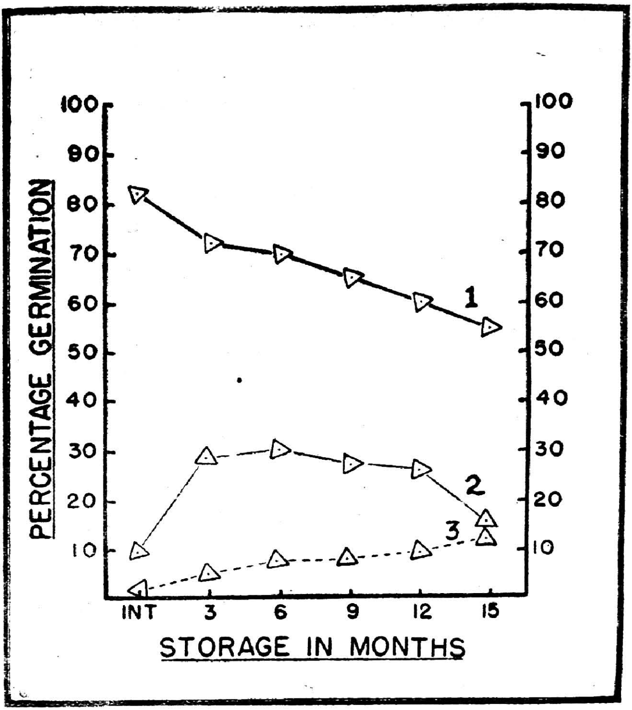


Text-fig. V. Showing the germination and infection of fuzzy seeds of K. 1 variety with 12 per cent. moisture content after different periods of storage at 10°C.

Percentage germination — Curve 1.

Percentage infection by fungi — Curve 2.

Percentage infection by bacteria — Curve 3.

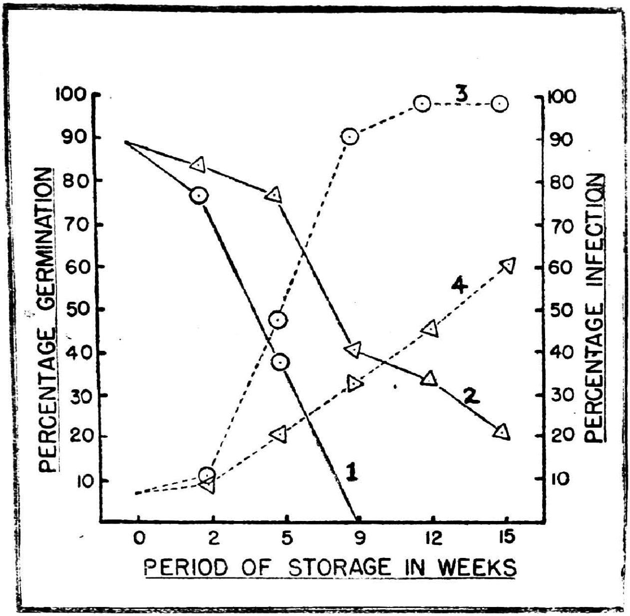


Text-fig. VI. Showing the germination and infection of delinted seeds of K. 1 variety with 12 per cent. moisture content after different periods of storage at 10°C.

Percentage germination — Curve 1.

Percentage infection by fungi — Curve 2.

Percentage infection by bacteria — Curve 3.



Text-fig. VII. Showing the percentage germination and infection of fuzzy and delinted cotton seeds of G. 1 variety with 13 per cent. moisture content after different periods of storage in sealed containers at 28°-30°C.

Germination of fuzzy seeds — Curve 1.

Germination of delinted seeds — Curve 2.

Infection of fuzzy seeds — Curve 3.

Infection of delinted seeds — Curve 4.

1. All seeds stored in open containers under storage temperatures of 28°-30°C. and humidity approximating 75 per cent were non-viable after 9 months, regardless of the internal moisture content of the seeds.

2. Both acid delinted and fuzzy seeds with an initial internal moisture content of 6 per cent retained their viability markedly better when stored in sealed containers than when stored in open containers at temperatures of 28° to 30°C. (Text Figs. I to IV).

3. Under sealed conditions of storage and similar internal seed moisture content, fuzzy seeds tended to lose their viability more rapidly than the delinted lot at 28° to 30°C., whereas the reverse was the case in open storage (Text Figs. I to IV and VII).

4. Seeds with high internal moisture content of 9 and 12 per cent lost their viability very rapidly both in sealed and in open storage (Table III).

5. Whereas fuzzy seeds with high internal moisture contents of 9 and 12 per cent retained their viability appreciably well up to 15 months' storage at 10°C. and 0°C., the corresponding lots of delinted seeds lost their viability very rapidly at these temperatures. The delinted sample with 12 per cent moisture content at 10°C. contained only 16 per cent viable seeds after 15 months' storage and the lot stored at 0°C. contained no viable seed after 12 months' storage. Thus high internal seed moisture content was found deleterious to delinted seeds in retention of viability at low temperatures i.e., 10°C. and 0°C. (Table II).

6. Fuzziness was found to be a factor which assumed an important role in the retention of seed viability at low temperatures (Text Figs. V, VI).

7. Fuzziness, on the other hand, contributed to loss of seed viability at storage temperature of 28° to 30°C.

8. Low temperature, combined with fuzziness and sealed storage were factors found to be essential for safe storage of seeds of any moisture content over the period tried in this experiment.

9. Positive correlation existed between fungal infection of non-viable seeds and loss in viability by the seed lot with high moisture content when stored at temperature of 28° to 30°C. in either open or sealed containers (Table III).

10. Under ordinary storage conditions fungi, both externally and internally to the cotton seed, tended to lose their viability after 9 months' storage ; no fungal infection was manifest in sealed storage at laboratory room temperature (Text Figs. II and IV).

11. Fungal infection of either fuzzy or delinted seeds was not co-extensive with the loss of viabiilty of the seed lot at low temperatures of storage, 10°C. and 0°C.

12. The onset of loss of viability by cotton seeds was associated with the production of abnormal seedlings (Table I).

Experiment II.

The previous experiment showed that cotton seeds with high moisture content lost their viability very rapidly and such lots contained a high percentage of seeds infected with fungi and bacteria. These results, however, left unanswered the question as to whether the loss of viability of these seeds was due primarily to infection by micro-organisms since the time interval between the samplings was as large as three months and hence the progressive role of micro-organisms in seed deterioration could not be followed closely. As only a negligible percentage of the seeds contained fungal and bacterial infection at the beginning of the experiment, it was apparent that seed infection in storage was caused by the external microflora—probably borne on the surface of the seed. The question mainly arose as to how seed viability will be affected if the entry of the micro-organisms into the seed from without is prevented by the application of fungicides to the seed.

Cotton seeds of H. 1 variety (*Gossypium herbaceum*) were divided into two lots, one lot being delinted, whereas the other was retained as normal fuzzy seeds. Portions of these two lots were adjusted to 9, 12·5 and 15 per cent. moisture content. One portion at each of these moisture levels from both the fuzzy and delinted lots was then divided into two samples, one sample being treated with 2 per cent. Agrosan G., while the second sample was left untreated. All seed samples were stored in glass stoppered bottles with tight lids to prevent fluctuations in moisture content. Germination and infection tests were made after 3, 5, 9, 12 and 15 weeks' storage at a temperature of 28°-30°C. Results are presented in Table IV.

TABLE IV — Showing the percentage germination and infection of fuzzy H. 1 seeds (*Gossypium herbaceum*) with 12·5 per cent. moisture content stored for different periods at 28°-30° C.

Period of storege	Untreated seeds				Treated with 2 per cent. Agrosan G.			
	Viable : non-viable seeds	Percentage infection of non-viable seeds			Viable : non-viable seeds	Percentage infection of non-viable seeds		
		Total	Fungi	Bacteria		Total	Fungi	Bacteria
Initial	98 : 2	0	0	0	98 : 2	0	0	0
3 weeks	72 : 24	10	4	6	76 : 19	10	0	10
5 weeks	43 : 50	30	8	22	49 : 45	26	0	26
9 weeks	34 : 54	46	8	38	39 : 54	41	0	41
12 weeks	12 : 73	54	12	42	13 : 68	62	0	62
15 weeks	0 : 100	77	19	58	0 : 100	78	0	78

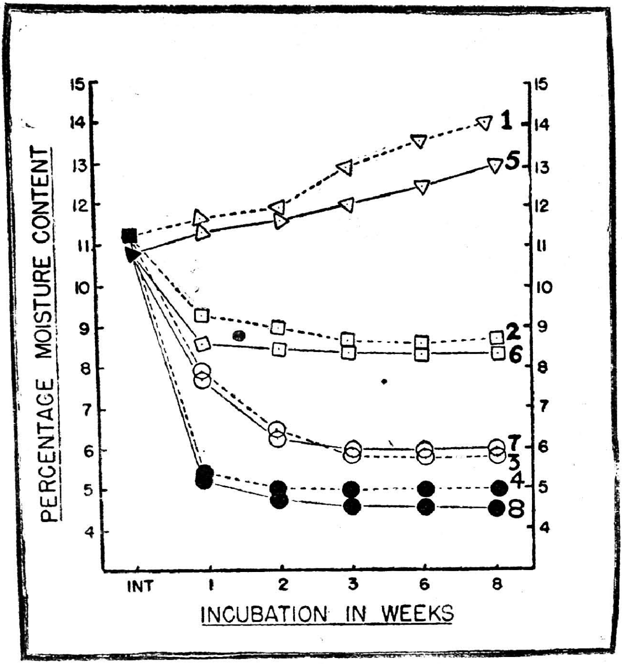
1. Treatment of seeds with the fungicide did not influence the loss of seed viability in storage.

2. Since none of the non-viable seeds in the sample treated with the fungicide showed any fungal infection, the results point out to the insignificant role of fungi in seed deterioration in storage.

That internal moisture content of cotton seeds is a cardinal factor in determining the longevity of the seed was amply demonstrated in previous experiments. According to Simpson and Miller (1944) cotton seeds rapidly attain moisture equilibrium with the surrounding atmosphere. The relationship, therefore, between the humidity of the external atmosphere and the internal moisture content of indigenous cotton seeds was studied.

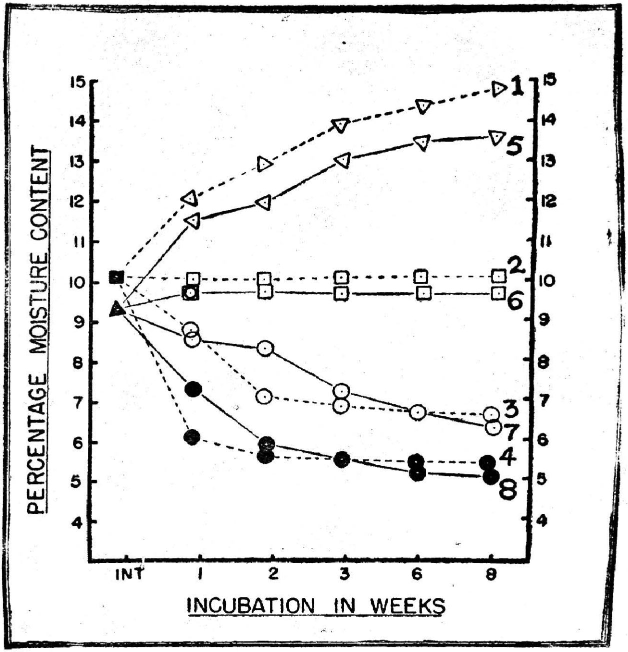
Experiment III.

One year old cotton seeds of Karunganni variety (*Gossypium arboreum*) and CO·2 strain (*Gossypium hirsutum*) were divided into two lots, one lot being retained as normal fuzzy seeds while the other lot was acid delinted and sun dried. The initial moisture contents of the seeds of the four lots were determined and these placed in desiccators over strengths of sulphuric acid necessary to maintain relative humidities of 35, 50, 75 and 95 per cent. Data of Wilson (1921) were used in preparing the acid solutions. Since a change in the moisture content of the seed would result in a change in the concentrations of the acid over which the seed was stored, the original acid in the desiccator was replaced with fresh



Text-fig. VIII. Showing the rate of moisture absorption by fuzzy and delinted seeds of CO·2 strain *(Gossypium hirsutum)* after storage at different relative humidities.

Delinted seeds at 95 per cent. humidity — Curve 1.
Delinted seeds at 75 per cent. humidity — Curve 2.
Delinted seeds at 50 per cent. humidity — Curve 3.
Delinted seeds at 35 per cent. humidity — Curve 4.
Fuzzy seeds at 95 per cent. humidity — Curve 5.
Fuzzy seeds at 75 per cent. humidity — Curve 6.
Fuzzy seeds at 50 per cent. humidity — Curve 7.
Fuzzy seeds at 35 per cent. humidity — Curve 8.



Text-fig. IX. Showing the rate of moisture absorption by fuzzy and delinted seeds of Karunganni variety (*Gossypium arboreum*) after storage at different relative humidities.

Delinted seeds at 95 per cent. humidity — Curve 1.
Delinted seeds at 75 per cent. humidity — Curve 2.
Delinted seeds at 50 per cent. humidity — Curve 3.
Delinted seeds at 35 per cent. humidity — Curve 4.
Fuzzy seeds at 95 per cent. humidity — Curve 5.
Fuzzy seeds at 75 per cent. humidity — Curve 6.
Fuzzy seeds at 50 per cent. humidity — Curve 7.
Fuzzy seeds at 35 per cent. humidity — Curve 8.

acid after 3 weeks. The seeds in desiccator were incubated at temperature of 28°C to 30°C. Moisture determinations of the seed samples were made after 1, 2, 3, 6 and 8 weeks' incubation and results are incorporated in Text-figs. VIII and IX.

1. The rate of absorption of moisture by seeds of Karunganni variety was higher than that by seeds of CO·2 strain.

2. In almost every case, the percentage moisture content of delinted seeds was found to be higher than that of fuzzy seeds after 8 weeks' storage.

3. Fluctuations in the moisture contents of seed were much more marked in delinted lots than in fuzzy samples.

4. A relative humidity above 75 per cent would increase the moisture content of seeds of Karunganni cotton above 10 per cent which in previous experiments proved to be injurious to cotton seed viability at ordinary temperatures.

B. Seed-borne fungi.

Experiment IV.

A comprehensive study of the internal microflora of the cotton seed was made employing thirteen different seed lots (obtained from the Provincial Agricultural Department and from self pollinated plants grown in the experimental plots of this laboratory) in isolation tests as described earlier. In addition, seeds collected from both healthy and diseased cotton plants in 'wilt' infected fields of the Udamalpet cotton growing tracts were also examined to evaluate the status of *Fusarium vasinfectum* — the causal organism of cotton wilt — as a seed-borne fungus. Parallel with the studies on the internal microflora of cotton seed, the fungi occurring on the exterior of the seed were isolated by plating out unsterilized fuzzy seeds in sterile Petri dishes over moist filter paper under aseptic conditions. Results are presented in Table V.

1. The fungus flora of the interior of cotton seeds comprised of mostly common saprophytic forms. Species of *Fusarium* and *Colletotrichum* were not isolated from any of the seeds tested, although these two genera have been commonly reported by other investigators.

2. None of the seeds collected from cotton plants in 'wilt' infected fields as well as the other samples tested, yielded *Fusarium*

TABLE V—Showing the percentage occurrence of different fungi within cotton seeds collected from various sources.

Cotton seed variety	Source	Percentage seeds yielding fungi						
		Total	*Aspergillus.* spp.	*Penicillium* spp.	*Rhizopus* spp.	*Fusarium* spp.	*Alternaria* spp.	Other fungi
K. 1	Department of Agriculture, Madras	39	30	8	3	0	1	2*
K. 4706	do.	46	40	11	3	0	0	0
K. 5090	do.	40	31	13	1	0	3	0
G. 1	do.	31	26	7	1	0	2	0
N. 14	do.	22	13	9	2	0	2	0
H. 1	do.	19	12	9	1	0	1	0
CO. 2	do.	23	14	12	0	0	3	0
CO. 3	do.	35	23	21	1	0	2	0
CO. 4	do.	32	20	21	2	0	3	0
K. 1	Plants grown in the Laboratory experimental plots.	63	58	7	2	0	1	2**
K. 4706	do.	69	59	10	2	0	3	2**
Pb. 72	do.	51	40	11	1	0	2	2**
CO. 2	do.	21	10	13	1	0	1	0
Karunganni cotton	Plants growing in wilt infected fields of Udamalpet.	16	14	4	1	0	0	0

** *Rhizoctonia bataticola.* * *Trichoderma* sp.

vasinfectum on culturing, thereby indicating the improbability of this fungus being disseminated through the agency of cotton seed, borne as an internal infection.

3. Species of *Aspergillus, Penicillium* and *Rhizopus* were isolated as common seed concomitants together with a few instances

recorded of *Trichoderma* sp. and *Alternaria* sp. infecting the interior of the cotton seed.

4. *Macrophomina phaseoli* (Maubl.) Ashby (*Rhizoctonia bataticola* (Taub.) Butler) was for the first time isolated from a small percentage of cotton seeds borne as an internal infection.

Experiment V.

All isolates of fungi occurring both externally and internally to cotton seeds were tested for their pathogenicity on cotton with a view to evaluating the part played by these fungi in pre-and post-emergence infection of cotton when introduced as infection foci into the soil substratum. In addition, some forms of soil fungi isolated from infected cotton seedlings were also included in the pathogenicity trials.

Purified cultures of the fungal isolates were inoculated in 200 g. of sterilized soil-oatmeal medium (prepared by autoclaving seived soil, powdered oatmeal and water in the ratio of 9:1:3 at 20 lbs. pressure for two hours) in suitable Erlenmeyer flasks and incubated at laboratory room temperature 23°C. to 30°C. for three weeks. Inocula of different isolates prepared by this method were then added to sterilized composted soil in glazed porcelain pots of 6″ × 9″ size; to every 1800 g. of the sterilized soil 200 g. of the inoculum was added. Six pots containing the soil inoculum mixture were run for each of the isolates, the soil moisture level being maintained at 65 per cent of the total moisture holding capacity of the soil. In another set of six pots with sterilized soil, 200 g. of sterile oatmeal medium was added, mixed thoroughly and incubated to serve as controls. Acid delinted surface sterilized seeds of Karunganni cotton were then sown in all the pots in the following way: 200 g. of the soil from each of the pots were first removed and the soil surface made even. Sixteen of the sterilized seeds were then placed in the pots, equidistant from one another and covered with the 200 g. of the soil. In all 96 seeds were sown in each set of six pots inoculated with each of the fungal isolates. All the pots were incubated in the glass house and studies on germination and infection of seeds were made upto 30 days after sowing. Results are incorporated in Table VI.

TABLE VI — Germination and infection of cotton seeds in sterilized soil artificially infected with various fungi.

Fungal inoculum	Isolated from	Percentage germination of seeds after 15 days	Percentage pre-emergence	Percentage of seedlings infected after		Percentage stand of healthy seedlings
				15 days	30 days	
Fusarium vasinfectum	Exterior of cotton seeds and infected cotton seedlings	15	75	11	15	0
Fusarium moniliforme	Diseased cotton seedlings	64	26	33	46	18
Fusarium sp. (3)	Exterior of cotton seed	61	29	20	27	34
Fusarium sp. (5)	do.	85	5	0	0	85
Fusarium sp. 7	do.	60	30	24	37	23
Rhizoctonia bataticola	Interior of cotton seed	58	32	33	41	17
Rhizoctonia solani	Cotton seedling	0	90	0	0	0
Pythium sp.	do.	50	40	12	21	29
Rhizopus nodosus (Strain A)	Interior of cotton seed	49	41	16	18	31
Aspergillus ustus	do.	86	4	0	0	86
Aspergillus niger	do.	86	4	0	0	86
Aspergillus fumigatus	do.	60	30	17	39	21
Aspergillus nidulans	do.	51	39	8	12	39
Aspergillus flavus	do.	88	2	0	0	88
Aspergillus terreus	do.	87	3	0	0	87
Alternaria sp.	do.	84	6	0	0	84
Trichoderma sp.	do.	85	5	0	0	85
Penicillium sp. (1)	do.	89	1	7	14	75
Penicillium sp. (2)	do.	85	5	0	0	85
Control		90	0	0	0	90

93187

1. Since species of *Fusarium* were generally most prominent in isolations from diseased seedlings grown in sterilized soil and from unsterilized cotton-seeds, they were regarded probably as among the most important seed-borne pathogens also responsible for poor emergence.

All isolates of Fusaria produced a considerable percentage of pre-emergence infection of seeds and in particular *Fusarium vasinfectum* was very severe both at the pre-and post-emergence stage. None of the seedlings in pots inoculated with *F. vasinfectum* survived after 30 days. In the series inoculated with *Fusarium moniliforme* although the percentage pre-emergence was of slight consequence, damping-off of the seedlings occurred soon after emergence and a high percentage of seedlings succumbed within 30 days. Further the seedlings were etiolated and the examination of the infected seedlings revealed numerous brownish lesions on the collar region in milder attacks, which in the severe manifestations of the disease had extended to the entire root system of the seedling.

2. *Macrophomina phaseoli* was pathogenic to a considerable extent in seed germination and produced a high percentage of post-emergence infection of the seedling.

3. The two forms *Rhizoctonia solani* and *Pythium* sp. (isolated from diseased cotton seedlings in unsterilized soil) were responsible for considerable dimunition of seed germination in soils inoculated with these isolates ; none of the seeds germinated in pots inoculated with *R. solani.*

4. Many of the common saprophytic forms of fungi — isolates from within the cotton seed — displayed considerable potentialities in reducing germination and emergence of cotton seeds and producing damping-off of seedlings. *Aspergillus fumigatus* and *Aspergillus nidulans,* respectively, produced 30 and 39 per cent of pre-emergence of cotton seeds in soil and 39 and 12 per cent of damping-off of seedlings. *Penicillium* sp. (1) although producing negligible pre-emergence infection, damped off 14 seedlings within 30 days.

5. *Rhizopus nodosus* Namyslowski recorded for the first time as occurring in the interior of the cotton seed reduced germination to a considerable extent and infected 18 of the seedlings on emergence in soil. The post-emergence infection of the seedlings was manifest by the attack of the cotyledons by the fungal mycelium, the latter showing profuse growth and sporulation on the cotyledons. In certain cases the fungus infected the cotyledons

soon after emergence resulting in the rapid decay of the seedlings. On examination of the ungerminated seeds in such soils artificially infected with *Rhizopus nodosus,* fungal ramification was observed around the seeds. Further, the fungus made free and profuse growth in unsterilized soil.

Experiment VI.

Studies on the pathogenicity of *Rhizopus nodosus* Namyslowski were made in detail with particular reference to soil conditions and the parasitic activity of this organism on cotton seed and seedling. This fungus by virtue of its ability to make free growth and perennate freely in unsterilized soil falls into the category of *Macrophomina phaseoli* observed to make similar free growth in unsterilized soil substrata by Kovoor (Unpublished), of *Rhizoctonia solani* observed by Blair (1943) and of *Pythium aphanidermatum* by Sadasivan (1947).

500 g. of seived black cotton soil were taken in 7″ × 6″ earthenware pots and one set of the pots sterilized at 20 lbs. pressure for two hours. The two different sterilized and unsterilized series of pots were further divided each into four sub lots. In all 10 pots were run for every sub-lot. The four sub-lots of both the series were then inoculated in the following way: in the first, 100 g. of the soil were removed from each of the 10 pots, 10 seeds of Karunganni variety placed on the soil surface equidistant from one another and 10 cotton root bits infected with the fungus placed in juxtaposition with each seed. The seeds were then covered with the 100 g. of soil. In the second set, cotton seeds were similarly sown in the 10 pots and each pot watered with 100 ml. of spore suspension of the fungus obtained by shaking sterile water in flasks with one week old culture of the fungus growing on standard potato dextrose agar medium. In the third series, 100 g. of the soil from each of the 10 pots were removed, 10 cotton seeds sown equidistant from one another and 2 agar discs of the fungal colonies placed besides each seed and the latter covered with the 100 g. of the soil. The agar discs were cut from one week old fungus colonies growing on standard potato dextrose medium in Petri dishes. In the fourth sub-lot, cotton seeds were sown in the pots in a similar manner and the pots inoculated with 10 g. of cotton seed meal inoculum, obtained from one week old culture of the fungus growing in the cotton seed meal. The moisture content of the soil in all the pots was adjusted to 60 per cent of the total moisture holding capacity. Ten pots sown with 10 seeds each and uninoculated were run as controls. The entire experiment was

carried out under glass house conditions and observations made 3, 5, 7, 10 and 15 days after sowing. Results are incorporated in Table VII.

TABLE VII — Showing percentage germination and infection of cotton seeds sown in sterilized and unsterilized soils infected by different inocula of *Rhizopus nodosus* (strain A).

Inocula of the fungus	Percentage germination of seeds after 15 days		Total number of seedlings infected by the fungus	
	Sterilized series	Unsterilized series	Sterilized series	Unsterilized series
Infected root bits ..	47	54	3	2
Spore suspension ..	70	73	1	0
Agar discs ..	47	53	4	4
Cottonseed meal ..	33	39	8	6
Control ..	74	72	0	0

1. Marked reduction in percentage germination of the seeds was caused by the inoculation of pots with cotton seed meal culture of the fungus.

2. Inoculation of the soil with spore suspension was found to be innocuous. The other two forms of inocula i.e., infected root-bits and agar discs reduced germination of cotton seeds to almost the same extent.

3. With the exception of spore suspensions, the other three forms of inocula reduced germination of seeds to a considerable extent in both sterilized and unsterilized soil. Thus sterlization of soil to delimit microbial antagonism did not delimit the pathogenic capacity of *Rhizopus nodosus*.

Two distinct strains of *Rhizopus nodosus* Namyslowski were differentiated by their growth character in potato-cane sugar medium. Whereas one strain exhibited good vegetative growth and sporulation the other strain made feeble growth although sporulating freely. Further on culturing these two strains in potato-dextrose medium, no differentiation in growth characters were observed. The growth behaviour of the two strains were studied in potato-cane sugar medium adjusted at different pH levels ranging from 2 to 9.

Experiment VII.

50 ml. of potato-cane sugar medium were taken in 250 ml. Erlenmeyer flasks and six such flasks were adjusted to each of the following pH levels: 2·0, 3·0, 4·0, 5·0, 6·0, 7·0, 8·0, 9·0. The flasks with the medium were previously sterilized at 15 lbs. pressure for 10 minutes and pH adjustments made using sterile standard hydrochloric acid and sodium hydroxide under aseptic conditions. The media were inoculated with cork borer discs of the two strains (strain A and strain B), 3 flasks at each of the eight pH levels being maintained for each of the two strains. The flasks were incubated at temperature of 27°C. to 29°C. for four days after which the fungal mats were filtered off, washed, dried and weighed. pH of the filtrate was also tested and results are presented in Table VIII.

TABLE VIII — Showing the growth of *Rhizopus nodosus* (strain A and B) at different pH levels in potato-cane sugar liquid medium.

Initial pH of the medium	Dry weight of the fungal mat in mg. (after 4 days growth)		pH of the filtrate	
	Strain A	Strain B	Strain A	Strain B
2·0	Nil	Nil	—	—
3·0	126 mg.	135 mg.	2·25	2·25
4·0	151 mg.	142 mg.	2·35	2·35
5·0	100 mg.	149 mg.	7·55	2·55
6·0	70 mg.	174 mg.	8·05	2·65
7·0	36 mg.	148 mg.	8·15	2·68
8·0	34 mg.	109 mg.	8·25	2·72
9·0	Nil	Nil	—	—

1. Strain B tolerated a wide range of pH levels, whereas strain A made very spares growth at pH levels above 5·0.

2. Strain B accumulated maximum dry weight at pH 6·0 and made good growth at a wide range of pH levels from 3·0 to 7·0, whereas strain A attained maximum growth at pH. 4·0 and evinced poor growth at the other pH levels above 4·0.

3. Strain B lowered the pH of the medium considerably: pH of all the filtrates ranging from 2·25 to 2·72. Strain A on the other

hand showed marked variations. The filtrate of the media originally at pH 3·0 and 4·0 were found to have pH values of 2·25 and 2·35 respectively. However the final pH of the filtrates of the medium originally at pH 5·0 and 6·0 was found to be 7·55 and 8·05 respectively.

The two physiologically different strains of *Rhizopus nodosus* Namyslowski were further experimented upon on their capacity to produce disease reaction in soils of different hydrogen-ion concentrations.

Experiment VIII.

500 g. of black cotton soil were added to 30 earthenware pots kept in 3 series. Pots in two series were then treated with different dilutions of commercial hydrochloric acid and saturated with water to facilitate the reaction of the acid with the soil. All pots were incubated for 3 weeks after which samples of soil were tested for pH. In both the series of pot treated with acid, the pH of soil was brought to 4 and 6·1, the pH of the soil left untreated being 7·8. Acid delinted surface sterilized cotton seeds of Karunganni cotton were then sown in all the pots and 10 pots in each of the series were inoculated with agar discs of *Rhizopus nodosus* (strain A and B). The other 10 pots were left uninoculated to serve as controls. In all 10 seeds were sown in each of the pots. Soil moisture in all the pots was maintained at 60 per cent saturation capacity and observations on germination of seeds and infection made after 3, 5, 7, 10 and 15 days' incubation at glasshouse temperature. Results are presented in Table IX.

TABLE IX — Showing percentage germination and infection of cotton seeds in natural soil adjusted at different pH levels and inoculated with *Rhizopus nodosus* Namysl. (Strains A and B).

Soil	Percentage germination of seeds after 15 days in soils inoculated with			Total number of seedlings infected after 15 days		
	Strain A	Strain B	Control	Strain A	Strain B	Control
4·0	34	53	68	3	1	0
6·1	49	58	70	4	0	0
7·8	55	60	72	3	1	0

1. Lowering of soil pH level markedly reduced the percentage germination of seeds in pots inoculated with strain A.

2. The pathogenic capacity of strain B in reducing the percentage germination of seeds in pots inoculated with the fungus was not influenced by the pH of the soil to any appreciable extent.

DISCUSSION

Simpson (1946) has stated the conditions governing the longevity of seeds of American Cotton. He observed that seeds of American cotton when stored in different places in the cotton belt retained their viability for 5-6 years at certain places, whereas in some case the seeds were non-viable within 1-2 years. In these studies, however, seeds of K. 1 variety with an internal moisture content above 9 per cent lost their viability within 9 months at laboratory room temperature of storage, thus bringing out the necessity of furnishing suitable conditions out of the ordinary for safe storage of seeds to any considerable extent. The only reports of some importance on the longevity of cotton seeds of indigenous varieties by Sonavne (1928, 1934) state that such seeds retain their viability for eight years, no appreciable loss in viability being noticed until after the third year. It must be mentioned, however, that the various environmental factors which influenced the storage experiments of Sonavne have not been recorded. Indeed the marked discrepancies existing in the results obtained by the American workers on the longevity of stored cotton seeds and between the results obtained in these studies and those recorded by Sonavne (1928, 1934) are probably attributable to the ' keeping qualities " of the seeds themselves and not to the storage conditions.

Among the various conditions influencing loss in viability of stored cotton seeds, factors of temperature and internal seed moisture content were of primary importance and interdependent. Generally, seeds of low moisture content (approximating 6 per cent) tolerated a higher range of storage temperatures up to 28°C. to 30°C. (laboratory room temperature), whereas temperatures of 10°C and 0°C. were necessary for retention of viability of seeds with higher moisture content of 9 and 12 per cent (Tables II and III). However, it was interesting to note that fuzzy seeds lost their viability more rapidly than the delinted lot at laboratory room temperature of 28°C. to 30°C., although the opposite was the case at temperatures of 10°C. and 0°C. when fuzziness was found to be a factor essential for safe storage of seeds ; delinted lot at low temperatures deteriorated rapidly (Table II). It is not known,

whether delinted seeds of other varieties and species will show similar reaction to cold storage and only further work with numerous other cotton varieties can bring out the extent to which these results can be generalized. Special emphasis is laid on the importance of sealed storage for safe keeping of seeds since indigenous cotton seeds were found to absorb moisture very rapidly under humid atmospheric conditions. In storage conditions with relative humidity at 75 per cent — by no means uncommon in the tropics — stored cotton seeds were found to contain more than 10 per cent internal moisture content (Text-fig. IX) thus resulting in rapid deterioration of seeds under ordinary storage conditions (Table III). Similar observation were made by Simpson and Miller (1944) on seeds of American cotton.

Although a direct correlation existed between the percentage infection of non-viable seeds and the rate of deterioration of seed samples under certain storage conditions, application of fungicide to the seeds with a view to warding-off fungal infection from without did not have any ameliorative effect on cotton seed viability, thus pointing out the unimportance of fungi in cotton seed deterioration in storage. This view is in conformity with the findings of Simpson (1946) and Arndt (1946 b). At present there is evidence to support the statement of Arndt (1946 b) that initial loss of cotton seed viability in storage is probably associated with autocatalytic processes within the embryo itself as described by Katon and Altschul (1944).

Investigations of Barre (1912), Lehman (1925), Ludwig (1925), Shands (1927), Dugger and Koehler (1944) and Arndt (1946 a) emphasized the importance of prolonged storage of seeds in order to eliminate certain of the seed-borne parasite which lost their viability before the seeds themselves under certain storage conditions. Similar observations were made in these studies when fungi on the exterior and interior of cotton seed were found to lose their viability under optimum storage conditions (Text-figs. II and IV). From the results in these investigations the following factors are considered optimum for safe storage of cotton seeds: (1) Internal seed moisture content not exceeding 6 per cent; (2) sealed storage; (3) storage temperatures of 10°C. to 0°C. for fuzzy seeds and 0°, 10° and 28° to 30°C. for delinted seeds.

Failure to isolate *Fusarium vasinfectum* from within the cotton seed (Table V) showed that this organism presumably played no part in disease dissemination from the interior of the cotton seed. The result corroborates the results of Neal and Gilbert (1935),

Ray and McLaughlin (1942) and others. Nevertheless, the isolation of *F. vasinfectum* and four other species of *Fusarium* (pathogenic to cotton) from the exterior of the cotton seed (Table VI) surmises the potential danger of sowing such seeds, thus resulting in increased infection foci in soil. Further, the internal microflora of cotton seed comprises of many saprophytic genera of which Aspergilli are the most prominent (Table VI). *Aspergillus ustus* (Bainier) Thom and Church, *Rhizopus nodosus* Namyslowski and *Macrophomina phaseoli* (Maubl.) Ashby, (*Rhizoctonia bataticola* (Taub.) Butler) were isolated from the interior of Indian cotton seeds for the first time.

Two of the most important isolates from the interior of cotton seeds, in these studies, were the two physiologically different strains of *Rhizopus nodosus* Namyslowski. It was interesting to note that strain A which did not tolerate a wide range of pH levels, had the ability to alter the initial acidic substratum of pH. 5·0 into a highly alkaline one (Table VIII). Strain A produced lesser mat weight than strain B and was generally more virulent as a pathogen (Table IX). Similar observations in the case of different strains *Fusarium bulbigenum* var. *lycopersici* — the tomato wilt pathogen — were recorded by Wellman (1942) who found that in a series of liquid media with a wide range of hydrogen-ion inconcentrations, cultures inoculated with virulent and mild strains showed that the former tended to induce alkaline reactions and the latter acid reactions.

SUMMARY

Studies were made on the influence of storage conditions on the loss of viability of indigenous cotton seeds together with the evaluation of the part played by micro-organisms in seed deterioration in storage. Parallel experiments were conducted to assess the internal microflora of cotton seeds and in particular the status of *Fusarium vasinfectum* — the cotton wilt pathogen —as a seed borne organism. The pathogenic reactions of seed-borne fungi on cotton seed and seedling were also studied.

1. Cotton seed with moisture content above 9 per cent lost their viability within 9 months when stored under ordinary conditions of temperature (28°-30°C.).

2. For safe storage of seeds to any appreciable extent, the following three factors were found essential (a) seed moisture content not exceeding 6 per cent (b) sealed storage and (c) storage temperatures of 0° and 10°C. in case of fuzzy seeds ; delinted seeds

tolerated a wider range of temperature from 0°C.-30°C. under these conditions.

3. Storage of delinted seeds with 6 per cent moisture content at 28°-30°C. proved optimum since the fungi infecting such seeds lost their viability earlier than the seeds themselves, the latter retaining their viability appreciably.

4. Fungi are not the primary cause of seed deterioration in storage.

5. *Fusarium vasinfectum* — the cotton wilt pathogen — does not seem to be seed-borne.

6. The internal microflora of cotton seeds comprised of mostly common saprophytic fungi among which Aspergilli were most prominent. *Aspergillus ustus* (Bainier) Thom and Church, *Rhizopus nodosus* Nawyslowski and *Macrophomina phaseoli* (Maubl.) Ashby (*Rhizoctonia bataticola* (Taub.) Butler) were isolated from interior of cotton seeds for the first time.

Most of the seed-borne fungi of cotton were capable of producing disease reaction when present as infection foci in soil and the potential danger of sowing such seeds is pointed out.

ACKNOWLEDGMENTS

The author is greatly indebted to Professor T. S. Sadasivan, M.Sc., Ph.D. (Lond.), F.A.Sc., Director, University Botany Laboratory, Madras, for suggesting the problem and offering ready guidance and helpful criticism throughout. Thanks are due to the authorities of the University of Madras for the award of a studentship during the tenure of which a part of this work was undertaken. Thanks are also due to the Director of Agriculture, Madras, for supplying the cotton seeds and to the Head of the Mycological Department, Indian Agricultural Research Institute, New Delhi, for the identification of fungal cultures.

REFERENCES

A. O. A. C. (1945) *Official and Tentative methods of Analysis of the Association of Official Agricultural Chemists.*

Arndt, C. H. (1946a) Effect of storage conditions on survival of *Colletotrichum gosssypi. Phytopath.* 36 (1): 24-29.

Arndt, C. H. (1946b) The internal infection of cotton seed and the loss of viability in storage. *Phytopath.* 36(1): 30-37.

Bain, Douglas C. (1939) Effect of sulphuric acid treatment on fungi and bacteria present in cotton seed from diseased bolls. *Phytopath.* 29: 879.

Barre, H. W. (1912) Cotton anthracnose. *South Carolina Agric. Expt. Sta. Bull.* 164.

Blair, I. D. (1943) Behaviour of the fungus *Rhizoctonia solani* Kühn in the soil. *Ann. appl. Biol.* 30 : 118-27.

Brown, A. H. (1933) Effects of sulphuric acid delinting on cotton seeds. *Bot. Gaz.* 94 : 501-503.

Crawford, R. H. (1923) Fungi isolated from the interior of cotton seed. *Phytopath.* 13 : 501-503.

Dastur, J. F. (1934) Cotton anthracnose in the Central Provinces. *Indian J. agric. Sci.* 4(1): 100-120.

Duggar, George H., and Benjamin Koehler (1944) Age of seed corn in relation to seed infection and yielding capacity. *J. Amer. Soc. Agron.* 36(5): 436.

Elliot, J. A. (1923) Cotton wilt, a seed borne disease. *J. agric. Res.* 23 : 387-393.

Garrett, S. D. (1936) Soil conditions and the take all disease of wheat. *Ann. appl. Biol.* 23 : 667-699.

Katon, M. L. and Altschul, A. M. (1944) Effect of moisture and treatments with acid and alkali on date of formation of free fatty acids in stored cotton seed. *Plant Physiol.* 19 : 310-325.

Keen, Bernard A. and Raczkowski, Henry (1921) The relation between the clay content and certain physical properties of a soil. *J. agric. Sci.* 11 : 441-450.

Kovoor, A. T. A. (1947) Soil conditions and root infecting fungi, with special reference to *Rhizoctonia bataticola* on cotton. (Unpublished).

Kulkarni, G. S. (1934) Studies in the wilt direase of cotton in the Bombay Presidency. *Indian J. agric. Sci.* 4 : 979-1048.

Lehman, S. G. (1925) Studies in treatment of cotton seeds. *Tech. Bull. N. C. agric. Exp. Sta.* 26, 71.

Ludwig, C. A., (1925) Studies with anthracnose infection in cotton seed. *S. C. Exp. Sta. Bull.* 222.

Muskett, A. E., and Malone, J. P. (1941) The Ulster method for the examination of flax seed for the presence of seed-borne parasite. *Ann. appl. Biol.* 28(1): 8-13.

Neal, D. C., and Gilbert, W. W. (1935) Cotton diseases and methods of control. *U. S. Dept. Agr. Fmrs'. Bull.* 1745.

Piper, C. S. (1944) *Soil and Plant Analysis.* Interscience Publishers, Inc. New York.

Rawlins, T. E. (1933) *Phytopathological and Botanical Research Methods.* N.Y.; John Wiley & Sons.

Ray, W. W., and McLaughlin, J. H. (1942) Isolation and infection tests with seed- and soil-borne cotton pathogen. *Phytopath.* 32 : 233-238.

Rudolph, B. A., and Harrison, G. J. (1945) The invasion of the internal structures of cotton seed by certain Fusaria. *Phytopath.* 35(7): 542-548.

Sadasivan, T. S. (1947) Saprophytic activity of *Pythium aphanidermatum,* the tomato wilt organism. (Unpublished).

Saran, A. B. (1945) On the viability of paddy seeds, *Oryza sativa. Curr. Sci.* 14(10): 271.

Shands, R. G. (1937) Longevity of *Gibberella saubinetti* and other fungi in barley kernels and its relation to the emetic effect. *Phytopath.* 27: 749.

Simpson, D. M. (1935) Relation of moisture content and method of storage to deterioration of stored cotton seed. *J. agric. Res.* 50(5): 449-456.

Simpson, D. M. (1942) Factors affecting the longevity of cotton seeds. *J. agric. Res.* 64(7): 407.

Simpson, D. M. (1946) The longevity of cotton seeds as affected by climate and seed treatments. *J. Amer. Soc. Agron.* 38(1): 32-45.

Simpson, D. M. and Miller, P. R. (1944) The relation of atmospheric humidity to moisture in cotton seed. *J. Amer, Soc. Agron.* 36: 957-958.

Simpson, D. M. and Stone, B. M. (1935) Viability of cotton seed as affected by field conditions. *J. agric. Res.* 50(5): 435-447.

Sonavne, K. M. (1928) Longevity of crop seeds. *Agric. J. India,* 23: 271-276.

Sonavne, K. M. (1934) Longevity of crop seeds. Part II. *Agri. and Live Stock in India* 4: 287-292.

Taubenhaus, J. J. and Ezekiel, W. N. (1932) Seed transmission of cotton wilt. *Sci., n.s.,* 76, 1959.

Tervet, Ian W. (1945) The influence of fungi on storage, on seed viability and seedling vigour of soyabeans. *Phytopath.* 35: 3-15.

Wellman, F. L. (1942) Differences in pH relations of some pathogenically variable strains of tomato *Fusarium. Phytopath.* 32: 271-288.

Wilson, R. E. (1921) Humidity control by means of sulphuric acid solution with critical correlation of vapour pressure data. *J. Ind. and Eng. Chem.* 13: 326-331.

THE DIGESTIBILITY AND AMINO ACID MAKE-UP OF CHEMICALLY MODIFIED KERATINS

BY

A. NARAYANASWAMY, PH.D.

(University Biochemical Laboratory, Madras)

A. *Introduction*

1. Keratins have been defined as 'proteins resistant to digestion by pepsin and trypsin, insoluble in dilute acids and alkalis, in water and in organic solvents, and yielding on acid hydrolysis histidine, lysine and arginine in the ratios of approximately 1:4:12'. (Block and Vickery, 1931). They are the products of epidermal activity and include the outer surface of the skin and such organs as scales, feathers, hair, wool, hoof and nails. The biological function of the keratins being to give the animal body mechanical protection and preserve it from bacterial attack, the insolublity and stability mentioned in the above definition is essential for their static function in the organism. The main interest to the biochemist of the keratins is the means by which these proteins achieve their insolublity and resistance to enzymes. The study of sheep's wool from this point of view was undertaken as it was expected to throw some light on (a) the structure of proteins, (b) the mechanism of the enzymic degradation of proteins. Wool was chosen for detailed study because of its economic importance in the textile industry and also because its purification presented no practical difficulties. It was considered desirable to devote particular attention to the factors which might increase its resistance to insect attack and to chemical reagents, especially alkalis, to which it might be exposed in the processes of bleaching or laundering.

2. *Present state of knowledge of the problem*:—The high cystine and sulphur content of keratins has been a matter of common knowledge for a long time, and has even been included in their definition (Goddard and Michaelis, 1934) so as to exclude silk fibroin and sericin from this group. The insolublity of the keratins has been attributed mainly to the cystine disulphide linkages occurring in them and supposed to link ladderwise the long peptide chains in the molecule. (Patterson *et al*, 1942). Evi-

dence for this comes from work on keratin derivatives obtained chemically, all of which have in common the property that the disulphide linkages have been destroyed wholly or in part by oxidation or reduction. The earlier papers dealing with such derivatives concern themselves mainly with oxidation methods. Lissizin (1928), Stary (1924, 25, 28) and Waldschmidt—Leitz and Schuckmann (1929) used bromine, permanganate and hydrogen peroxide as oxidizing agents, and the latter two obtained derivatives which were digestible by proteolytic enzymes. The oxidizing agents under the conditions used by these authors are not specific for the disulphide groups, but attack the protein molecule at other points also; moreover they act very slowly. The method of Stary especially who seems to have made a detailed study of the various fractions obtained by bromination suffers from the serious defect that the bromine is allowed to act in glacial acetic acid solution for a period of six weeks; and as no precautions were observed to dry the reagents used, the hydrogen bromide liberated during bromination would degrade the keratin to varying degrees, depending upon the amount of moisture present in the mixture. Actually, as will be seen from work done in the course of this investigation on the action of bromine, the bromination is complete in less than twenty four hours when dry reagents are used and there is practically no soluble degradation product.

The action of reductants on this class of proteins is of somewhat greater significance as with a proper choice of reagents and conditions, the disulphide group can be specifically reduced, at least in part, without affecting other points in the protein molecule. Reduced keratins were first studied in detail by Goddard and Michaelis (1934), who investigated the action of thioglycollic acid, potassium cyanide, sodium sulphite and sodium sulphide. The first three reagents act only in alkaline solution but sulphide is most active in neutral to mildly acid solution. Excepting in the case of thioglycollic acid the reaction is not simple and results in the introduction of extra sulphur or nitrogen, thus giving mixtures of products containing different amounts of nitrogen or sulphur. The thioglycollic acid reaction is a simple reduction and no introduction of sulphur occurs. With disulphides like cystine the reaction might be represented thus:

$$R{\cdot}S{\cdot}S{\cdot}R + 2\,HS{\cdot}CH_2COOH \rightarrow 2\,RSH + (S - CH_2COOH)_2$$

Unfortunately, under the conditions employed by Goddard and Michaelis, the yield of protein precipitated from the alkaline kerateine solution is not quantitative, there is destruction of fibre structure, and the unprecipitated portion is difficult to account for.

Otherwise great interest attaches to their preparation as it is readily digested by pepsin and trypsin and contains almost the same percentage of cystine as the original keratin.

Remarkably mild conditions have recently been worked out for the reduction of keratins with thioglycollic acid by Geiger, Kobayashi and Harris (1942). They carry out their reaction at pH 5 in a solution of calcium thioglycollate at 50°C and about half the cystine S — S linkages are reduced at one step in two hours. There is no destruction of fibre structure and the resulting product is enzyme digestible. Also, by alkylating the thiol group formed with alkyl halides of the types R·I and I·R_1·I (methylenic dihalides) they have been able to obtain two distinct types of derivatives, the former reagents yielding digestible products and the latter ones enhancing the stability of the resulting product towards enzymic attack. This provides very neat proof for the part played by the disulphide cross-linkage in the keratin molecule, as the methylenic dihalides might be supposed to promote the formation of — S — $(CH_2)_n$ — S — cross linkages which would be more resistant to disruption than the disulphide linkage itself. The rates of digestion of the kerateine derivatives have not been studied in detail, only physical methods having been employed to show the variations in resistance to enzyme action of the derivatives.

3. *Present Investigation: Scope and Purpose*:—As will be evident from the above review of existing knowledge of the problem, the relation of the stability of the keratins to their molecular structure has not been worked out exhaustively. The part played by the other side chain groups in the keratin molecule and their possible combinations in salt like, peptide or ether linkages as envisaged by previous workers on the structure of keratins (Speakmann and Townsend, 1936; Astbury and Street, 1932; Astbury and Woods, 1934) has not been investigated. The problem of oxidized keratins seemed to merit further study, especially with a view to devise suitable methods of oxidation involving minimum and measurable destruction of the basic and other side groups of the various amino acid residues, and yielding a homogeneous product. For this the uptake of bromine by wool keratin under anhydrous conditions had to be measured and the time course of the reaction worked out. This has been carried out under different sets of conditions and the products obtained analysed for their content of amino acids likely to be affected by the oxidative treatment, as also of artifacts resulting from such treatment. The rate of digestiblity has then been measured in as much detail as permitted by existing methods of analysis. The observations re-

garding reduction products of wool keratin of Geiger *et al* have been confirmed and preliminary measurements of their digestiblity made, though non-availablity of thioglycollic acid in sufficient quantities made it impracticable to study the problem in as great detail as would be desirable. Also, for purposes of comparison, and because the data from numerous X-ray studies of the keratins (Astbury, 1939) require confirmation by chemical methods of investigation, a preliminary study of the amino acid composition of wool was undertaken. During the course of this work on the amino acids of wool, certain modifications were sought to be introduced into the present methods of amino acid analysis; the preliminary removal of cystine by means of the cuprous mercaptide method of Lucas and Beveridge (1940) was studied in some detail and certain shortcomings of the technique which were observed have been confirmed by Bailey *et al* (1943) in a recent publication. This aspect of the matter is discussed in greater detail in the experimental section.

B. *Experimental*

1. *Purification of wool keratin* :—Native wool obtained from Messrs Bangalore Silk and Wollen Mills was separated from foreign matter by careful picking and then washed with alkali free soap. It was extracted with ether, benzene and alcohol in succession and again washed with several changes of distilled water. It was then dried in a warm current of air.

2. *Amino acid composition of wool* :—(a) *Hydrolysis* :—Purified wool in lots of 100 g. at a time was brought into solution in 6 N hydrochloric acid by heating on the water-bath, then refluxed on the air bath for 18 hrs. After hydrolysis, as much as possible of the acid was expelled by repeated distillation in vacuo, and after removal of a suitable aliquot for total nitrogen and other analyses, the solution was subjected to treatment with cuprous oxide as below. In certain cases, like the isolation and estimation of the basic amino acids 25% sulphuric acid was used for the hydrolysis, the acid removed quantitatively with baryta, and the barium sulphate precipitate washed repeatedly with boiling water so as to remove as much as possible of the adsorbed amino acids.

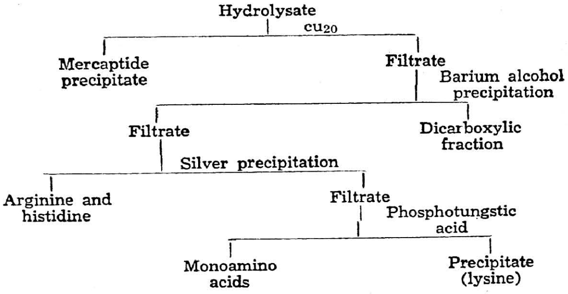
(b) *The removal of cystine as the cuprous mercaptide* :—The hydrochloric acid hydrolysate from 100 g. of wool after removal of most of the hydrochloric acid by evaporation in vacuo was diluted to 2 litres and a 10% suspension of cuprous oxide was added in a

slow stream with rapid mechanical stirring; the addition was stopped when a small test sample gave no further reaction with cuprous oxide and the supernatent was deep blue. The cuprous oxide was prepared by the reduction of Fehling's solution with glucose and washed with distilled water till free from alkali and then suspended in water. The stirring of the mercaptide precipitate was continued for some time longer and it was then left in the refrigerator overnight. The precipitate was separated by centrifuging and washed a number of times with distilled water. The preciptate was then suspended in water and decomposed by hydrogen sulphide with mechanical shaking. The sulphide preciptated was filtered and washed, and the combined filtrate and washings were concentrated in vacuo after removal of dissolved hydrogen sulphide by bubbling in air. The concentrate was made up to known volume, an aliquot removed for analysis, and the rest of the solution was concentrated further after bubbling in oxygen to oxidize most of the cysteine present; the substance separating out contained mostly cystine and was easily purified by one crystallization.

(c) *Treatment of the filtrate from the mercaptide precipitation*: — The filtrate from the mercaptide precipitation was also treated with hydrogen sulphide and all the copper removed as sulphide; the precipitated copper sulphide was washed thoroughly, and the filtrate and washings combined. The combined filtrate was then freed from hydrogen sulphide, brought to pH 4·5 and concentrated in vacuo, when most of the tyrosine separated out and was removed and washed with small quantities of cold water. The final concentrate was made up to known volume, aliquots removed for analysis and the dicarboxylic acids precipitated from the rest of the solution according to Foreman's method as modified by Damodaran (1931).

(d) *The amino acids in wool*: *Methods of Analysis*:—In the case of hydrolysates of wool, total nitrogen, amino nitrogen, ammonia nitrogen, and cystine, arginine and tyrosine were determined. Ammonia nitrogen was determined in the Parnas apparatus using a suspension of magnesium oxide and after removal of ammonia, the hydrolysate was made up to volume and used for amino nitrogen determination in the Vanslyke apparatus. Total nitrogen, amino nitrogen, cystine, arginine and tyrosine were determined on the mercaptide precipitate also, after removal of copper. During the concentration of the filtrate from this precipitation, the fraction precipitating out was analysed for total nitrogen, amino nitrogen and tyrosine by Zuverkulow's (Block, 1938) method and was found

to consist mainly of tyrosine. The concentrated filtrate was free from all humin and of a straw yellow colour, the cuprous oxide being quite efficient for the removal of humin and colouring matter. On the dicarboxylic acid fraction precipitated from this, total nitrogen, amino nitrogen and dicarboxylic acid nitrogen were determined after quantitative removal of barium, the amino nitrogen and dicarboxylic nitrogen being determined according to the titrimetic method of Harris (1929). Isolation of glutamic acid as hydrochloride and aspartic acid as the copper salt was also attempted. From the filtrate after precipitation of the dicarboxylic amino acids, arginine and histidine were precipitated at strongly alkaline pH according to Kossel and Kutscher's method (1901). The silver precipitate was treated with hydrogen sulphide and the fraction containing arginine and histidine analysed for total nitrogen etc. Arginine and histidine were also determined colorimetrically by the method of Sakaguchi and the diazo reaction respectively. From the filtrate after precipitation of arginine and histidine, lysine was precipitated as the phosphotungstate, total nitrogen and amino nitrogen being determined on this fraction, and after freeing it from phosphotungstic acid, the lysine was precipitated as the picrate. The final fraction obtained therefore contained the monoamino acids and the usual analyses for total nitrogen and amino nitrogen were made on this. The scheme adopted for the analysis might be represented thus:



3. *Comparative examination of wool and its brominated derivatives*:—The following determinations were made on wool and its bromination products, apart from the amino acid analysis already described: total nitrogen by the micro-kjeldahl method, total sulphur by Pirie's method (1932) of wet digestion with a mixture of nitric and perchloric acids in the presence of copper

and estimation of the sulphate obtained as barium sulphate. The hydrolysate of wool brominated in carbon tetrachloride and in water were also analysed for ammonia nitrogen and amino nitrogen by the usual methods.

Estimation of cystine, arginine, tyrosine and histidine: the colorimetric methods as described in Block (1938) were adopted throughout, the Sullivan reaction for cystine, the Sakaguchi reaction for arginine, Zuverkulow's reaction for tyrosine and the diazo reaction for histidine.

4. (a) *Preparation of reduced keratin*:—The recent method worked out by Geiger *et al* (1942) was used for reducing the disulphide linkages of wool. The thioglycollic acid was neutralized to pH 5 with calcium oxide while being kept cooled in ice, as otherwise the resulting solution became coloured. For every gram of wool 20 ml. of 0·3 N calcium thioglycollate was used and the reaction allowed to take place at 50°C for 2 hrs. Wetting of the wool was facilitated by evacuating the reaction vessel with a filter pump. The wool was removed at the end of 2 hrs. and washed several times with distilled water to remove thioglycollic acid and its oxidation products. The filtration was done as rapidly as possible and the reduced wool used for digestion experiments straightaway.

(b) *Reoxidation of the sulphhydryl groups of the reduced wool*:—Before the reduced wool was reoxidized, it was washed thoroughly with 95% ethyl alcohol as otherwise mixed disulphides of cysteine and thioglycollic acid are known to be formed. It was then rinsed with distilled water, placed in a 0·1M potassium phosphate buffer at pH 7 and a stream of oxygen bubbled through till the wool no longer gave a positive nitroprusside reaction. Finally the wool was again washed with distilled water and used for digestion experiments.

(c) *Alkylation of the sulphhydryl groups of reduced wool*:—The reduced wool was shaken mechanically with a 50% aqueous methanol solution containing ammonia and ammonium chloride to a concentration of M/20 and M/10 respectively; for each gram of wool 100 ml. of the above solution and 0·002 mole of methyl iodide was used. The shaking was continued till the nitroprusside reaction was negative and then the methylated wool was filtered off and washed several times with distilled water.

5. *The uptake of bromine by wool under anhydrous conditions*:—The bromine was shaken up with phosphorus pentoxide added in small quantities at a time in a stoppered cylinder kept

cooled in ice. Addition of phosphorus pentoxide was stopped when there was no further reaction and development of heat. The bromine was then filtered through glass wool. The glacial acetic acid used was freed from water by repeated freezing, pouring out the unfrozen supernatent and thawing the solid acid. 33 g. of dry bromine (10·5 ml.) was dissolved in 100 g. (94 ml.) of glacial acetic acid. 3 ml. of the solution was added to each gram of wool accurately weighed out into 12 stoppered bottles. The first day of the experiment 3 ml. of the bromine solution in acetic acid was made up to 100 ml. with ice water, and 10 ml. of this was added to 5 ml. of 15% potassium iodide, followed by 5 c.c. of 4 N hydrochloric acid and 25 ml. distilled water. The solution was titrated against standard thiosulphate using starch as indicator towards the end. On subsequent days one each of the stoppered bottles was washed out with ice cold water into a 100 ml. standard flask and the excess bromine estimated as above. This was done on every alternate day for three weeks and the results are tabulated. The experiment was repeated once again using 5 ml. of the bromine this time and the results obtained were similar as can be seen from the table.

6. *The preparation of brominated wool*:—(a) 100 g. of purified wool was shaken up with 100 g. of bromine dissolved in 300 ml. of carbon tetrachloride in a stoppered bolt head flask. There was development of heat on thorough mixing, and the mixture was then kept in the dark for 24 hrs., within which time the uptake of bromine was known from the above experiments to have ceased. It was then quickly filtered through paper, washed with 4 or 5 changes of carbon tetrachloride and finally with a small quantity of 95% alcohol. The carbon tetrachloride washings on evaporation in vacuo yielded no residue, but the washing with alcohol gave a small amount of solid matter. The brominated wool was then dried in a vacuum desiccator over sulphuric acid and potassium hydroxide sticks to remove the last traces of bromine, and could easily be broken up into a uniform powdery material. Under the microscope it still retained its fibre structure.

(b) Instead of carbon tetrachloride, glacial acetic acid was used in the earlier experiments for the bromination, the procedure being the same as above, glacial acetic acid being used for washing also; but it was found that with carbon tetrachloride the material could be more easily freed from the final traces of bromine and as the products were not likely to differ very much as regards points

of attack on the keratin molecule, carbon tetrachloride was used for all subsequent work.

(c) In aqueous solution wool was found to decolorize bromine quite rapidly and bromination in aqueous solution was expected to follow a somewhat different course from that in organic solvents, as in aqueous solution there would be greater likelihood of oxidative changes in the sidechains. 100 g. of wool was shaken up with saturated bromine water in a bolt head flask, the decolorized solution being decanted off every time fresh bromine solution was added. This was continued till there was no further decolorization even on standing, and the wool was let stand in the bromine solution for 2 hrs. It was then filtered off and washed with several changes of distilled water till there was no further bromine in the washings. The brominated wool was then dried as above in a vacuum desiccator over sulphuric acid and potassium hydroxide. This preparation could also be ground to a fine powder, but the material in this case was somewhat coarser. Under the microscope it was seen to be fibrous in structure.

7. *Measurement of the rate of digestion* :— The enzymes used for the digestiblity experiments were pepsin, trypsin and pepsin followed by trypsin. B.D.H. preparations of the enzymes were used throughout. For pepsin 5 g. of the modified keratin was shaken up with N/20 hydrochloric acid, brought to pH 1·8 and a volume of 240 ml. After being kept in the incubator for an hour 10 ml. of a suspension of 250 mg. pepsin was added. Similarly for trypsin 5 g. of keratin was brought to pH 8·3 and a volume of 240 ml. and after an hour in the incubator 10 ml. of a suspension of 250 mg. trypsin was added. In the case of tryptic digests the amino nitrogen liberation was measured every hour for the first six hours. For pepsin followed by trypsin 200 ml. of the peptic digest was brought to pH 7, boiled to destroy pepsin, cooled, brought to pH 8·3 and trypsin added, the volume being brought to 250 ml. The amino nitrogen was measured according to the titrimetric method of Harris (1929). 5 ml. aliquots were pipetted out and titrated against N/10 alkali to pH 6·5 using bromothymol blue as indicator. 1 ml. of neutralized formalin and 6 drops of 1% phenolphthalein were added to another aliquot and the titration carried to a pink colour. The difference was taken to be a measure of the amino nitrogen in the solution. For the calculation of percentage split, the modified keratin was hydrolyzed with acid and the amino nitrogen in an aliquot of the hydrolysate was measured by the same method. Thus the alkali equivalent to the total amino

nitrogen in the aliquots taken from the digests could be calculated and from this the percentage split.

8. *Tests for the diketo piperazine group in the brominated wools* :—One of the objections to Abderhalden's theory (1924) of the diketo piperazine linkage in protein molecules is the resistance of the diketopiperazines to the usual proteolytic enzymes. It was therefore thought necessary to investigate by means of the usual colour tests the possiblity of its occurrence in the native keratin molecule and possible destruction on bromination. The modified method of Brand and Sandberg was adopted for the test as in the original procedure according to Abderhalden and Komm (1924) substances containing sulphur give a yellow colour on boiling with sodium carbonate which masks the colour of the reaction. The test actually employed was carried out as follows: the substance to be investigated was boiled with 2—3 ml. of saturated sodium carbonate solution for about 1 minute and was immediately layered carefully with 0·5 — 1 ml. of a solution of the reagent, picric acid or dinitrobenzene. A distinct ring was formed in the case of a positive reaction.

C. *Results and Discussion*

1. *The amino acids of wool* :—The results of colorimetric estimations obtained in the course of our investigations is tabulated along with those of previous workers on the subject. The deviations are of interest in the case of arginine where we have consistently obtained higher values. For histidine, hydrolysates of our wool did not give a satisfactory value as the colour produced did not compare well with that of the standard even in the arginine histidine fraction.

NITROGEN AS % TOTAL NITROGEN

	Present investigation	Marston (1928)	Block (1939)
Cystine ..	8·89	8·59	9·93
Arginine ..	22·9	18·42	18·16
Tyrosine ..	2·1	2·08	2·26
Histidine ..	—	10·5	1·23

2. ***Analysis of wool*** :—

Ash	..	0·82%
Sulphur	..	2·70%
Moisture	..	13·04%
Nitrogen	..	14·92% (uncorrected)

3. *Analysis of HCl hydrolysate* :—

(Total nitrogen	..	7·65g)
Ammonia nitrogen	..	7·2%
Amino nitrogen	..	71·8%
Nitrogen in mercaptide precipitate	..	13·9%
Nitrogen in dicarboxylic fraction	..	24·5%
Amino nitrogen in dicarboxylic fraction		99·7% of nitrogen in the fraction
Amino nitrogen by titration	..	89·4% „ „
Nitrogen in arginine histidine fraction	..	21·3%
Nitrogen in arginine isolated as flavianate	..	13·7%
Nitrogen in histidine flavianate	..	2·1%
Lysine nitrogen (isolated as picrate)	..	3·5%
Mono amino nitrogen	..	26·2%
Amino nitrogen in monoamino acid fraction	..	83·6% of nitrogen in the fraction

4. *Analysis of brominated wool.*

	Wool brominated in carbon tetrachloride	Wool brominated in aqueous soln.
Total nitrogen	13·61%	14·51%
Total sulphur	2·03%	—
Ammonia nitrogen	12·8% T.N.	15·0% T. N.
Amino nitrogen	64·6% „	63·0% „
Cystine nitrogen	nil	nil
Tyrosine „	nil	nil
Arginine „	22·95% T.N.	17·95% T.N.

5. *Amino acid changes in brominated wool*: The amino acid groups likely to be affected by bromination are chiefly those of cystine, tyrosine and the basic amino acids. The disulphide group in cystine is known to be oxidized by the halogens to the sulphonic acid groups (Friedmann, 1902; Shinohara, 1932) and it was expected that cystine residues in the intact protein might

undergo similar oxidation. Tyrosine residues could be brominated in the nuclear part of the molecule and this seems to happen in the intact protein molecule from the evidence we have gathered from an analysis of the brominated keratin after hydrolysis. The destruction of basic groupings was studied in detail only in the case of the arginine residue and was found to take place to a negligible extent. The following is an account of the attempts made to ascertain the changes undergone by the above amino acid groupings during the course of bromination.

(a) *Cysteic acid* :—It was expected that due to its strongly acid sulphonic group, cysteic acid if present would be precipitated along with the dicarboxylic acids during Foreman's barium alcohol precipitation. To verify this the sulphur content of the dicarboxylic acid fraction separated in the usual way was determined by the method of Pirie (1932). It was found that 94·5% of the total sulphur of brominated wool could be recovered in the dicarboxylic acid fraction. Estimation of cystine by the Sullivan method showed that destruction of the amino acid was complete in wool brominated either in carbon tetrachloride or with saturated bromine water. Recoveries of added cystine from the hydrolysis was quite satisfactory. Attempts were made to isolate the cysteic acid from the hydrolysate basing our procedure on this fact. 20 gm. of brominated wool was hydrolysed with hydrochloric acid and after evaporation of most of the acid, the dicarboxylic fraction was precipitated and freed quantitatively of barium with sulphuric acid. The dicarboxylic acids were then evaporated down to a thick syrup with the addition of 96% alcohol to free it as much as possible from water and then boiled with a further quantity of alcohol. The insoluble portion was filtered off and the sulphur content of the fractions was followed as an index of their cysteic acid content. The alcohol extract was found to contain all the sulphur and was evaporated down and taken up in a small quantity of water. This was extracted in a continuous extractor of the Kutscher — Steudel type with ethyl acetate. The extract was found to contain no sulphur and on evaporation yielded some tarry matter; it was also found to contain most of the bromotyrosine formed during bromination. Attempts to crystallize out the cysteic acid from the final fraction, either as the free acid using alcohol or as the basic copper salt were not successful as the dicarboxylic amino acids seemed to react very similarly to cysteic acid. The analytical values obtained in the course of the investigation are listed below :—

	Alcohol insoluble fraction	Ethyl acetate extract	Cysteic acid fraction
Sulphur (Cal. as cysteic acid)	nil	nil	446 mg.
Total nitrogen	21·9 mg.	56·3 mg.	635 mg.
Amino nitrogen	84·8% T.N.	72·7% T.N.	87·8% T.N.
Xanthoprotein test	Negative	Strongly positive	Negative

(b) *The fate of tyrosine on bromination*:—The expected bromination product of the tyrosine residue was 3: 5 dibromotyrosine whose properties have been exhaustively studied by Mörner (1913). The hydrolysate of neither of the brominated wools answered Million's test for tyrosine and estimation of tyrosine by Zuverkulow's method gave zero values for the amino acid, thus showing that it was completely brominated. According to Mörner dibromotyrosine gives the xanthoprotein reaction and does not give a colour with Millon's reagent. This was actually found to be the case with our hydrolysates. Also, dibromotyrosine is known to be precipitated along with the dicarboxylic acids and it was noticed that the dicarboxylic fraction from our hydrolysates gave a very strong xanthoprotein reaction, whereas the filtrate from this fraction answered the test only feebly. The solublity of dibromotyrosine in organic solvents was expected to be greater than that of ordinary amino acids due to the halogen atoms in its molecule and actually the substance responsible for the xanthoprotein reaction was found to be quantitatively extracted by ethyl acetate. The ethyl acetate extract also answered the chlorine water test of Mörner, giving a turbidity on addition of chlorine water, which disappeared on shaking up with ether. But in view of the very small quantity of brominated tyrosine present in the keratin derivative, no attempt was made to isolate it.

(c) *Arginine*:—Arginine was estimated by the Sakaguchi reaction of the hydrolysate and also on the phosphotungstate precipitate. The values were in close agreement except for the fact that in the case of the hydrolysate of wool brominated in water, there was a fluorescence developing on addition of alkali and the colours did not compare well. Expressed as % of the total nitrogen, the arginine nitrogen was—

Wool brominated in carbon tetrachloride ..	22·95%
Wool brominated in aqueous solution ..	17·95%
Wool (native) ..	22·9%

It will be seen that there was practically no destruction of arginine when wool was brominated in carbon tetrachloride and the loss in the case of wool brominated in aqueous solution was not considerable, because the filtrate from the aqueous bromination of wool contained about ⅛ of the total nitrogen and of this 7·5 % was found to be arginine nitrogen.

6. *Course of bromine uptake by wool under anhydrous conditions* :— (Unreacted bromine as ml. N/10 thiosulphate ; 1 gm. of wool for each experiment).

Initial titre of bromine solution used	9·9 ml	14·7 ml
3rd day	2·05 ml	6·27 ml
5th day	1·80 ml	5·93 ml
7th day	1·80 ml	5·92 ml
9th day	1·80 ml	5·92 ml
..	..	..
..	..	..
21st day	1·80 ml	5·92 ml

7. *The filtrate fraction from the aqueous bromination of wool*:— Aliquots were hydrolysed with hydrochloric acid and total nitrogen and amino nitrogen determined on the hydrolysate. Arginine, cystine and tyrosine were estimated by the usual colorimetric methods and the digestiblity of the fraction by pepsin and trypsin tried after destroying the small amount of bromine present with sulphurous acid solution. The digestion could not be followed titrimetrically as the solution was too deeply coloured. Amino nitrogen was also determined on the fraction before hydrolysis.

Total nitrogen	1·749 gm	(11.7% of nitrogen in the wool brominated)
Amino nitrogen (before hydrolysis)	19·52% T.N.	
Amino nitrogen (after hydrolysis)	66·8% T.N.	
Free amino nitrogen in the filtrate fraction	28·2%	
Arginine nitrogen	7·5% T.N.	
Cystine „	nil	
Tyrosine „	nil	
Pepsin	digestible	
Trypsin	resistant	

The arginine estimation was not quite satisfactory as the hydrolysate developed a green fluorescence on addition of alkali.

8. *Analysis of the mercaptide precipitate* :—The following data are typical of those obtained in our precipitation experiments ;—

Total cystine in hydrolysate	**5·19g (·606g nitrogen)**
Nitrogen in mercaptide ppt.	**1·587g**
Cystine in mercaptide ppt.	**5·10g**
Arginine	**Present**
Tyrosine	**Nil**

Though the separation of cystine was quantitative there was much extraneous nitrogen, and arginine formed an appreciable proportion of this. Bailey (1943) has obtained similar results in his recent detailed investigation of the method, though in his experiments arginine does not seem to be a constituent of the mercaptide precipitate. In view of these findings the technique of the precipitation requires further investigation and improvement.

9. *The diketopiperazine reaction of the derivatives* :—

Substance tested	Reagent used	
	Picric acid	Dinitrobenzene
1. Wool	Strongly + ve	Strongly + ve
2. Wool brominated in carbon tetrachloride	+ ve	+ ve
3. Wool brominated in aqueous solution	Strongly + ve	Strongly + ve

It was felt however that the very strong reaction given by native wool might be due to the fact that sulphur was split off from wool on boiling with sodium carbonate, as observed by Jones *et al* (1941) in their work on lanthionine. To test the effect of the sulphide so formed, the reaction was tested also on the following substances :—

Substance tested	Reagent used	
	Picric acid	Dinitrobenzene
1. Sodium sulphide	— ve	+ ve
2. Ammonium polysulphide	Strongly + ve	Strongly + ve
3. Cystine	No immediate ring formn., red colour on mixing.	
4. Edestin	— ve	— ve

It is highly suggestive that polysulphides react so positively, as the sulphur split off from keratin might dissolve in the alkali to give polysulphide. In any case the positive reaction of cystine and sulphides stress the need for caution in attaching too great significance to this test, especially as edestin which is of low cystine content reacts negatively.

10. *Digestiblity of reduced keratin and its derivatives*:—

(a) *Hydrolysis of reduced keratin and its derivatives by pepsin.* (expressed as % split)

No. of days	Reduced keratin	Methylated keratin	Reoxidized kerateine
1	1·5	2·3	1·8
2	2·2	4·0	3·1
3	3·0	5·5	4·0
4	3·5	6·6	4·8
5	4·2	7·4	5·4
6	4·4	8·0	5·9
7	4·5	8·1	6·5

(b) *Hydrolysis of reduced keratin and its derivatives by trypsin* (expressed as % split)

No. of days	Reduced keratin	Methylated keratin	Reoxidized kerateine
1	8·7	14·5	12·0
2	12·8	19·0	16·2
3	15·9	22·2	19·7
4	18·5	25·2	22·5
5	20·6	27·5	24·4
6	22·0	28·2	25·7
7	22·5	28·5	26·2

It will be seen that the methylated keratin digests fastest, a finding in agreement with the results of Geiger *et al* (1942) who have studied only the digestion by pepsin and that by physical methods of stress strain measurements which are not quite satisfactory for following the course of an enzyme reaction. The results obtained

in the case of reoxidized kerateine is somewhat unexpected and requires more detailed verification.

11. *The digestiblity of brominated wool* :—

(a) *Hydrolysis of brominated wool by pepsin*
(expressed as % split)

No. of days	Wool-brominated in carbon tetrachloride	Wool brominated in in aqueous soln.
1	9·8	6·3
2	10·7	7·1
3	11·8	8·0
4	12·7	8·7
5	13·6	9·3
6	13·8	9·5
7	13·9	9·5

(b) *Hydrolysis of brominated wool by trypsin*
(expressed as % split)

No. of hours	Wool-brominated in carbon tetrachloride	Wool brominated in in aqueous soin
0	13·9	9·5
1	18·9	10·8
2	22·1	12·7
3	25·0	12·9
4	26·3	13·7
5	27·2	14·1
6	28·1	14·3
24	34·7	16·9
48	36·9	18·7
72	37·8	19·4
96	38·4	19·8
120	38·5	19·8
144	38·5	19·9

With both pepsin and trypsin, wool brominated in carbon tetrachloride digests faster than wool brominated in aqueous solution. The course of digestion in the case of wool brominated in aqueous solution is somewhat irregular, but otherwise the two derivatives behave like the ordinary digestible proteins both towards

pepsin and trypsin. The oxidative changes in aqueous bromination seem somehow to retard digestion, as otherwise the two brominated derivatives do not differ much in the side chain destruction they have suffered. The preparation obtained by aqueous bromination gave a positive reaction with potassium iodide and starch, though it had been washed free of all bromine. This suggests the presence of perbromide linkages which are known to inhibit proteolytic digestion.

D. *Summary*

Methods for the bromination of wool under anhydrous conditions and in aqueous solution have been worked out, the factors likely to cause disruption of the keratin molecule being reduced to a minimum. The effect of such bromination on the residual groups of the amino acids has been investigated, with particular reference to cystine, tyrosine and arginine. For purposes of comparison the amino acids of wool have also been analysed in some detail. The diketopiperazine test has been applied to all the preparations and the results obtained show the unsatisfactory nature of the test when applied to keratins. The rates of digestion of brominated wool of reduced wool, and derivatives obtained from reduced wool have been measured in the case of pepsin and trypsin.

The author wishes to thank the Madras University for the award of a studentship during the years 1942-44 when the investigations reported here were carried out.

REFERENCES

1. Abderhalden, E., & Komm, E. (1924). Z. Physiol. Chem., *134*, 121.
2. Astbury, W. T., & Street, A. (1932). Phil. Trans. Roy. Soc. Lond., A. *230*, 75.
3. Astbury, W. T. & Woods, H. J. (1934). Ibid., *232*, 333.
4. Astbury, W. T. (1939). Ann. Rev. Biochem., *8*, 113.
5. Bailey, K., Chibnall, A. C., Rees, M. W., & Williams, E. F. (1943), Biochem. J., *37*, 361.
6. Block, R. J. (1938). The Determination of Amino acids, Minneapolis.
7. Block, R. J., & Vickery, H. B., (1931). J. Biol. Chem. *93*, 113.
8. Brand, E., & Sandberg, M. (1926). J. Biol. Chem., 70, 381.
9. Chibnall, A. C., Rees, M. W., & Williams, E. F. (1943). Biochem. J., *37*, 372.
10. Damodaran, M. (1931). Biochem. J., 25, 2123.
11. Friedmann, E. (1902). Chem. Zentr. Bl., VI, 2, 1360.
12. Geiger, W. B., Kobayashi, F. F., & Harris, H. J. (1942). J. Research. NBS, *29*, 381.
13. Goddard, D. R., & Michaelis, L. (1934). J. Biol. Chem., *106*, 605.
14. Harris, L. J. (1929). J. Biol. Chem. *84*, 296.

15. Horn, M. J., Jones, D. N. B. & Ringel, S. J. (1941). J. Biol. Chem., *138*, 141.

16. Kossel, A., and Kutscher, F. (1900-1), Z. Physiol. Chem., *31*, 165.

17. Levy, M. (1934), J. Biol. Chem., *105*, 157.

18. Lissizin, T. (1928), Z. Physiol. Chem., 173, 309.

19. Lucas, C. C. & Beveridge, J. M. R. (1940). Biochem. J., *34*, 1356.

20. Mörner, C. T., (1913). Z. Physiol. Chem., *88*, 138.

21. Patterson, W. I., Geiger, W. B. Mizell, L. R., & Harris, M. (1941). J. Research. NBS., *27*, 89.

22. Pirie, N. W. (1932). J. Biol. Chem. 96, 2044.

23. Shinohara, K. (1932). J. Biol. Chem., *96*, 285.

24. Sorensen, S. P. L., (1907). Biochem. Z. 7, 45.

25. Speakmann, J. B . & Townsend, F. (1936). Trans. Farad. Soc., 32, 897.

26. Stary, Z. (1924). Z. Physiol. Chem., *136*, 160.

27. Stary, Z. (1925). Ibid., *144*, 147.

28. Stary, Z. (1928). Ibid., *175*, 178.

29. Waldschmidt-Leitz, E., and Schuckmann, G. (1929), Ber. dtsch. Chem. Ges. *62*, 1891.

SYNTHETIC DETERGENTS AND DETERGENCY

BY

P. B. JANARDAHANAM, B.Sc. (Hons.)

Lecturer, Alagappa Chettiar College of Technology, Madras

The work of the last decade is reviewed with more than a hundred references. The present views on the mechanism of detergency with special reference to solubilization, effect of pH, and added electrolytes have been discussed. The present status and future trends of the detergent industry are pointed out.

The subject of detergents or of cleaning compositions is one of primary importance to society, since in every civilized circle cleanliness is considered next only to Godliness. The definition of a detergent seems to be easy and at the same time difficult. The American Society of Testing Materials defines it as "any material that cleanses". According to this, water, with its universal solvency, aided by mechanical action must be a good detergent. But, as Rideal[1] points out, this definition becomes quite inadequate when we have to distinguish between particulate matter probably held by mechanical forces, and molecular particles whose adhesion involves physico-chemical or electrostatic forces. It is with the latter type of soils that the detergents are exclusively concerned. They perform their function by (a) a process of deflocculation or detachment of the dirt from the substrate and (b) emulsification to prevent the redeposition of the detached dirt. The process of deflocculation involves wetting of the surfaces, and emulsification involves interaction between the soil and the detergent. McBain[2] describes a detergent as one which suitably charges the surface of the substrate so as to wet it thoroughly, and interacts physico chemically with the dirt so detached to remove it without redeposition, this latter process involving solubilization, suspension, emulsification, and foam-flotation, either individually or in various combinations. With the foregoing facts in view, we may here put down that detergents are essentially agents which even in low concentrations possess remarkable powers to modify the respective relationship between the gas/liquid, liquid/liquid, and liquid/solid systems at the interfacial layers.

Need for the Detergents :—Soap is the most important and all-purpose detergent whose history extends back into the hoary past.

The detergency of soap varies with pH; below pH 10 soap begins to decompose and lose its detergency. Another great drawback of ordinary soap is its chemical reaction with polyvalent cations with consequent formation of insoluble and non-detergent soaps. Though the latter disadvantage could be overcome to some extent by the use of builders like calgon, yet it weighs heavily against the use of soap in the hard water areas, one pound of soap being wasted per 100 gallons of approximately 10 degrees hard water.

The polar group of the soap, $-COONa$ is susceptible to hardness and the first attempts were to substitute this group so that the resultant product would be stable in hard water. With the help of a few organic unit processes like chlorination, hydrogenation, esterification, sulphonation and sulphation a series of products were brought into being which started the history of the so-called detergents, the better types of which possessed one or more of the following properties:—(a) Excellent wetting ability; (b) neutrality in solution; (c) solubility and effectiveness in cold water; (d) ready miscibility; (e) resistance to hard water, acids and alkalies; (f) good foaming properties; (g) tastelessness etc. As Boland[3] points out hardly any one detergent possessed all these coveted properties together, and to that extent very few could be designated as efficient detergents.

Though stray compounds possessing detergent properties had been prepared since the beginning of the century, industrial production was undertaken only in 1930 in Germany. Before and during the Second World War Germany was manufacturing at three centres[4] namely Witten (Ruhr), Oppau, Ludwig shafen, and Heyder bruk, substances like "Mersolates", "Gardinol" and "Igepon" (all alkyl sulphate type) by sulpho-chlorinating the Fischer-Tropsch hydrocarbons. In 1940 England and America started production of these detergents, and the war-time shortage of fats and oils, the essential raw materials of soap, gave a fillip to the synthetic detergent industry.

Strictly speaking, soap which is also a synthetic, produced from native fatty materials deserves the leading place in this class. But it is classed by itself to distinguish it from the 'Synthetic Detergents' which are got from synthetic hydrocarbons besides fatty materials. It is also interesting to note that at least one of the synthetic detergents occurs native in fruits or nuts, e.g. Saponin and attempts have been made to extract and utilize it for washing purposes.[5–7]

Nomenclature :—Hundreds of synthetic detergents are now competing on the retailer's shelves to capture the soap market. Though the various colourful names that confront the customer's eyes scarcely give any insight into the efficiency or the composition of the detergents, yet it will be to the gratification of his curiosity to know the regularity, if any, in the host of names that are coined.

(a) Some companies keep the same generic names for totally different classes of products e.g. :—Penetrol 60 and Penetrol 65.

(b) Some others use letters to denote the states like solution or powder e.g. —Nacconol N. R. of the National Aniline Co., and Mirapon R. K. of the Miranol Chemicals Ltd.

(c) Some use numbers or letters to designate the modification of the same basic chemical, as Span 20, and Span 40 of the Atlas Powder Co.

(d) In some cases the number following the names denotes strength. Areskap 50, Areskap 100.

(e) Sometimes the same composition appears under different names due to different companies manufacturing the same product. e.g. — Duponol, Olvus, Aurinol, denote the same product.

Classification :—The large number of these detergents, made possible by the several subtle variations in the composition, necessitate some sort of classification for the aid of the scientist who wants to investigate and improve them, and the industrialist who has to make a proper selection for specific purposes.

In 1938 C. V. Patel[8] described a classification in relationship to the practical use and based on the chemical nature. In 1940 Linsenmeyer announced in Melliand Textilber[9] a classification of detergents into 8 groups. The Science of detergency was not thoroughly investigated by that time and hardly could these classifications be concise and perfect. The present classification,[10-13] based on the ion which contains the lipophilic (oil-loving) group categorizes the detergents into Anionic, Cationic, Non-ionic and Zwitter ion types.

Soap with the structure of RCOO Na ionizes as $RCOO^- + Na^+$ and is typical of anionic detergents. All synthetic anionics have a similar pattern of construction so that when they ionize the lipophilic group falls in the negative radical.

Paice[13] has given an elucidative scheme of classification for detergents. The anionics may be considered under the following heads :—

(a) Sulphated oils with the general structure of

$$R_1\text{—}CH\text{—}R_2\text{—}COONa.$$
$$|$$
$$OSO_2\text{—}ONa$$

The presence of the —COONa group makes the product susceptible to hard water. K. G. Naik and Desai[14] have studied the sulphation and the sulphonation of a number of Indian oils like castor, peanut, safflower, mowrah, cotton-seed oils etc., and concluded that the stability of the detergent towards hard water increases with the percentage of the organic sulphonate group and the effective length of the hydrocarbon chains.

(b) *Alkyl Sulphates*:—Reduction of oil and fats by high pressure hydrogenation or by sodium metal yields long chain fatty alcohols which when sulphated yield detergents of the type $R\ CH_2 \cdot O \cdot SO_2 \cdot ONa$. The possibility of synthesizing these alcohols from inexpensive sources like kerosene[15] has very much cheapened these products in the market. The surface activity of this class of compounds has been investigated by Dreger et al.[16]

Corresponding to the normal alkyl sulphates there are also secondary alkyl sulphates got either from synthetic alcohols or by direct sulphation of olefines. The former are short chain bodies like the Tergitols of the Cynamide corporation[17] while the latter are long chain 'TEEpols'. The general formula of secondary sulphates is $R\text{—}CH\text{—}CH_3$ and herein as in the case of sulphated

$$|$$
$$OSO_2\text{—}ONa$$

oils[15–18] it has been shown that the sulphate group must be nearest to the end of the molecule to evince maximum detergency.

(c) *Alkyl Esters and Amides*:—Snell[19] has pointed out that these are the bases of a good many detergents. Some of the successful and highly selective wetting agents are the di-esters of the sulpho succinic acid, e.g.:—Aerosol type

$$R_{alkyl}\ OOC\text{—}CH\text{—}SO_3Na$$
$$|$$
$$R_{alkyl}\ OOC\text{—}CH_2$$

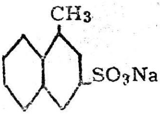
German Igepon A is a product of oleic acid and hydroxy ethane sulphonate, and has a formula $R\text{—}CO\text{—}OCH_2\text{—}CH_2\text{—}OSO_2Na$, Igepon T is a long chain fatty acid condensed with methyl taurine

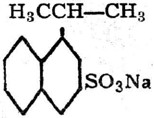
$$CH_3$$
$$|$$

and has the formula $R - CO - N - CH_2 - CH_2 - SO_2ONa$, and represents an amide-sulphonate type of detergent. Mirapon

RK is a sulphated amide. The use of carboxy amides as wetting agents in textile processing has also been investigated by Shirolkar.[20]

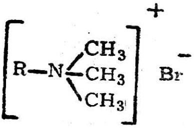
(d) Parallel with the development of aliphatic sulphates, there was the evolution of another series of compounds, namely alkyl aryl sulphonates. Patent literature will reveal the existence of a large number of these compounds. Work has been done on this class of anionics,[21-22] with a view to their utilization as textile auxiliaries. Methyl and isopropyl naphthalein sulphonates like

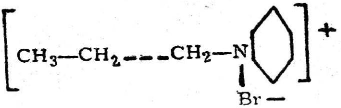
'Nekal 

and Perminal W 

already in the market. These are much better detergents than any previously described. Long chain-substituted benzene or phenol sulphonates like Naccanol, and Santomerse also fall in this category and possess alround wetting and detergent properties. The price of these are reported to be less than their naphthalene counterparts, and they account for most of the synthetics now available in U.S.A.

(e) *Sulphonated monoglycerides and poly-glycol esters*:— These compounds are popular in the food industries. Though industrial production of monoglycerides by directed inter-esterification of glycerides is still in the experimental stage, a good number of patents have already been taken on the glycol and glyceride-sulphonate-compositions as detergents.[25-27]

Cationic Detergents:—These are typified by Cetyl Trimethyl ammonium bromide 

and Cetyl pyridinium bromide 

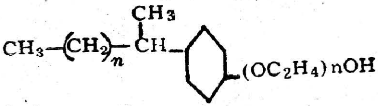
Here the Lipophylic group forms part of the positive ion and hence the name cationic.

These are acid stable unlike the anionics and soap, and therefore find utility in specific instances especially, where pH is below 10, eg., in a dyebath. Good accounts of their utility as textile auxiliaries, for disinfection, cosmetic manufacture, dyeing industry etc., have

been presented by various authors.[28-31] Generally they are slightly acidic in nature and have been shown[32] to be not compatible with soaps or anionics.

Besides being detergents, cationics serve as antiseptics and bactericides. Borghetty[33] commends cationics as very good softening agents that impart a soft 'handle' to the cotton fabric. Some of the popular commercial cationics are Triton X 400 of the Rohm and Haas, B. T. C. of Onyx Co., and the Arquads of the Armour Co.[34]

Non-Ionics:—These have the general formula of (a) CH_3—$(CH_2)_n$ $(OC_2H_4)_n$ OH, typified by Emulphon which is a condensation product of oleyl alcohol and ethylene oxide; and (b)

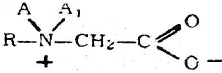


typifiel by Triton X 100,

which is got by condensing isobutyl phenol with ethylene oxide. The value of n• will easily be 10—12; and by varying the position and number of the ethylene oxide groups a large variety of compounds can be produced. A gradual transition from agents overbalanced in the polar direction to agents overbalanced in the non-polar direction can be obtained.

A review of the chemical research and discussion of some physical properties of non-ionics have appeared recently.[35-36] McBain[37] and Gonick[38] have presented data showing that nonionics form essentially micelle in solutions and in fact their detergency is not due to ionization but to this micelle formation, and due to a proper balance between certain hydrophilic and lypophilic groups.[39] An interesting method of preparing nonionics from low-melting wax acids and the relative efficiency of these products compared with the regular poly ethylene-glycol esters have been discussed by Griesinger.[40]

Zwitter ion type of detergents:—These are N-substituted amino acids of general formula



where A and A_1, are heterocyclic radicals,[39] or H, alkyl, or aryl groups.

The Science of Performance of Detergents:—In spite of the vast literature that has accumulated by way of investigation of surface activity, no clear picture has yet emerged of the nature

and sequence of the detergent action. Apart from the mechanical work that aids in the knocking out of the particulate soil, there are other factors required to remove the molecular soils attached to the substrate by physico chemical forces. In his SCI Medal address, Snell[41] pointed out that the soils adhere to the substrate mainly through three types of bonds, namely: (a) loose mechanical adherence, (b) strong ionic or Hydrogen-bonds as in the case of iron rust or ferric oxide pigments which are generally charged, and (c) by virtue of an adhesive layer, usually a fatty matter. Irrespective of the type of bond, whether it is to mechanically loosen the dirt or to neutralize the ionic charges or to displace the oily adhesive layer, the first essential requisite of any detergent is understood to be a wetting of the surfaces. The second function of the detergent is to deflocculate the detached dirt, and emulsify the same to prevent redeposition.

Scott and Roebuck[42] have attempted to elucidate the mechanism of wetting and penetration in relation to the chemical structure of the detergent molecule. During the penetration, the substrate/oil/soil interface must be replaced by the substrate/solution/soil interface ; and the smaller the energy associated with the new interface the more readily will it be formed. The interfacial energies are reduced by the detergents as per Gibb's theorem, owing to (a) migration of the detergent molecules to the surface, and (b) a characteristic orientation of the molecule at the interface. Preston[43] has attributed the driving force, causing the migration of the surface-active agent to the surface layers, to the free energy associated with the interface between the hydrophobic portion of the molecule and the surrounding water. The tendency of the molecule to orient at the surface layers can be attributed to a torque arising out of a united action of the water-attracting polar and the waterrepelling non-polar centres which occur in the same molecule.

Having thus seen how the surfaces are wetted by the detergents by diminishing the interfacial free energy, the next process is the detachment of the soil from the substrate. McBain[2] has explained this as a process of substitution, or ion exchange.

$$\text{Fabric-Dirt} + \text{Soap} \rightleftharpoons \text{Fabric-Soap} + \text{Dirt-Soap}$$

This ion exchange theory is supported by the fact that salts like Calgon which are added as builders to the detergents, though themselves devoid of detergency, have been known to be effective ion-exchangers. Another fact is that it is often extremely difficult

to remove the last traces of soap from the fabric after the washing operation, which indicates a compound formation between the fabric and the soap as per the above equation.

Gruntfest and Young[44] have plotted the amount of dirt in a system against the critical concentration of soap (i.e., the concentration above which the cloth is almost perfectly white and below which it is quite black). The linear characteristic of this curve (Fig. 1)

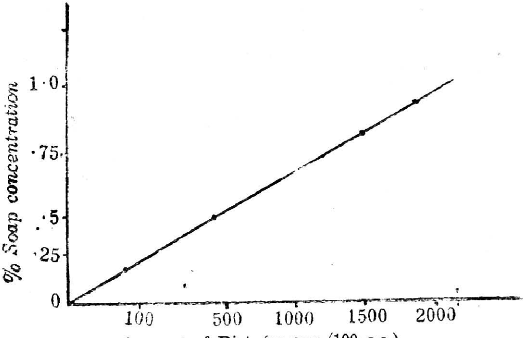


Fig. 1. Critical Soap Concentration Vs. the amount of Dirt.

indicates that the interaction between the soil and the soap is stoichiometric, as shown by the equation above. But the zero intercept may indicate that the fabric by itself hardly combines with the detergents. Snell[41] however favours the view that there is a definite sorption compound formation between the soap and the fabric.

All good detergents are also good wetting agents, but the reverse is not true. That all wetting agents need not be detergents has been shown by a simple demonstration experiment by Lomas.[45] What goes to make a good detergent is a combination of wetting power with dispersive power.

A study of the surface tension-concentration curves[2] of a few popular detergents show that the surface tension is at first drastically reduced, sometimes to a third of the value, sometimes passing through a minimum and then remaining constant with further addition of detergent, though the washing efficiency may just begin to increase only then. Hence dispersive power, which is the main cause of the washing efficiency, must be attributed to factors other than surface tension, factors like foam-flotation, emulsification, and solubilization. Of these, solubilization provides, by far, the

most important single factor. McBain has reviewed the various mechanisms postulated by different workers to explain the phenomenon of solubilization. Of these the most important is the formation of and the role played by detergent micelle.

It has been pointed out earlier that when a detergent dissolves in water, its molecules migrate and get concentrated at the surfaces. The hydrophobic group repelled by the water, shows a tendency to coalesce, and this tendency is proportional to the size of the hydrocarbon residue. The forces that oppose this coalition are the ionic repulsions between the polar hydrophilic ends of the molecules. For every hydrophilic group there is therefore, a limiting size of the hydrophobic group, beyond which coalescence and precipitation will take place. In between stage of complete solution and of precipitation, an intermediate stage of micelle formation can therefore be pictured.

Figure 2 from Preston's[43] data, illustrates the relationship between detergency, surface tension, and critical concentration for micelle formation. It will be noted that the detergency improves but little by further additions of detergent, when once the critical washing concentration is reached. This critical washing concentration coincides remarkably with the critical concentration for micelle formation, which in turn corresponds to the lowest surface tension value. The lowest surface tension value at the point when marked detergency starts, indicates that maximum wetting precedes the commencement of detergent action. If it is otherwise, proper detachment of dirt would not have taken place.

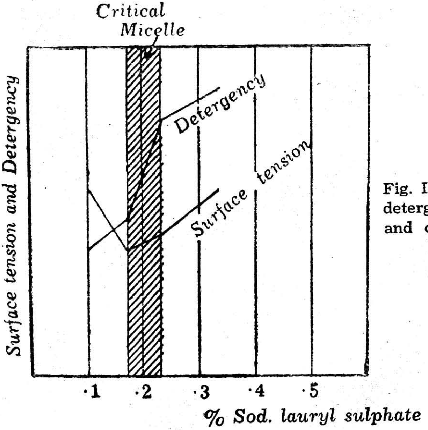


Fig. II. Relationship between detergency, surface tension, and critical miscelle concentration.

Now, does the coincidence of critical washing concentration with critical concentration for micelle formation mean that the formation of micelle is essential for detergency ? The answer could have been a bold 'YES' but for an observation of Gruntfest and Young. If we turn to Fig. 1, the zero intercept implies that the micelle formation is not essential to achieve efficient washing results. If it were, the intercept would be expected at the critical micelle concentration of respective detergents.

Micelle Formation and Solubilization :—Hartley [46–47] who was one of the earliest workers on the solutions of paraffin chain salts, assumed a spherical shape for the micelle, with about 50 hydrocarbon chains close-packed without any clearance and with the polar groups lying on the exterior, the diameter of the micelle being not greater than twice the length of the hydrocarbon chain. The interior of the micelle is in a liquid state and can dissolve oil or fatty soils in amounts comparable with an equal weight of free hydrocarbons. According to McBain spherical micelle cannot exist, since considerable geometrical difficulties will be encountered in such an arrangement, the soap molecules being 35 times as long as they are thick. Further the dimensions of such a micelle do not agree with the observed conductivity and the surfacedensity charge. The discovery, by X-ray diffraction, of a characteristic long spacing which decreases with increasing concentration, appears to favour a different picture. McBain suggests a lamellar micelle, in which the chains lie parallel to one another, and the outer faces of the arrayed ionic groups are separated by a definite thickness of water. When these lamellae act upon the soil, they expand and wedge the soil between the array of the hydrocarbon chains. Thus Kiessig and Philippoff[48] have shown by X-ray diffraction that when benzene is solubilized by sodium oleate, the thickness of the lamella increases from 91 A° to 127 A°. The lamellar view of micelle has been supported, with slight modifications, by the work of Ralston.[49]

The lamellar view of the micelle is however not perfect. Firstly, Bernal[50] has pointed out that in order to account for all the water in the system of soap solution, lamellae must be assumed to be separated laterally by a greater depth of water than that held between the ionic faces. And Harkins[51] has observed that when oil is dissolved in the soap micelle, the increase in the long spacing is much greater than is directly accounted for by the additional volume inside micelle.

A recent paper by Vetter[52] on the properties of the solutions of sodium salts of sulphonates (Aerosol M.A.), supports Hartley's early suggestion of a spherical micelle, except that the micellar

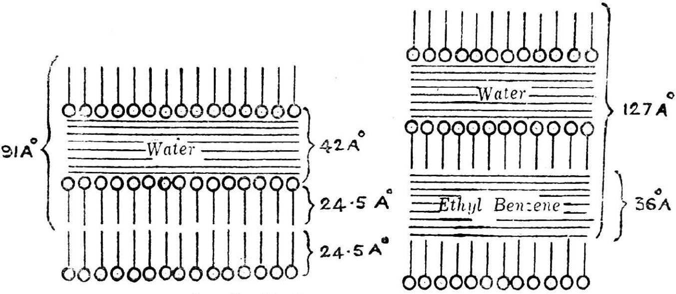
volume appears to be larger than that postulated by Hartley. Moreover in a note by Hartley in 'Nature',[53] he slightly modifies the hypothesis of exclusive solution in the micellar core, and admits the expansion of the spherical micelle to create free space to enclose some free oil or soil.

It is possible that both types of micelle exist, the lamellar structure in the more concentrated solutions. We may also note that there is a general agreement as regards the solubilization role of micelle, namely dissolution and entrainment of the fatty soil within the hydro-carbon chains. When polar compounds like fatty acids, fatty alcohols, and dyes are solubilized, it appears that unlike the case for the non-polar substances like ethyl benzene, the mechanism involves the incorporation of the solubilized molecules with their long axes lying almost parallel to those of the detergent molecules;[54] and consequently no increase in the long spacing (distance between the adjacent layers) can be observed by X-ray analysis on account of polar materials solubilized, when once the concentration for maximum colloidal form of the detergent has been reached.

Along with solubilization or incorporation of the dirt within the micelle, there is the possibility of sorption of dirt particles on the colloidal micelle. But McBain and McBain[55] consider this improbable as the hydrophobic soil particles should, as a rule, tend to be incorporated within the micelle, rather than on the outside in such large numbers as to render them unstable.

The Effect of Added Electrolytes on the Activity of Detergents:— When inorganic electrolytes are added to the synthetic detergent solution, the effect is to increase the dispersive power due to a closer packing of the molecules adsorbed on the surface. The closer packing is brought about by the ions of opposite charge reducing the repulsion between the charged polar heads of the detergent molecule.[47] Further addition of electrolyte will tend to flocculate the detergent, thereby considerably lowering the dispersive power. NaCl is found to cause this increase followed by a decrease, vide Fig. III. In the case of $Na_3\ PO_4$ the flocculating effect of the sodium ions is offset by the peptizing effect of the trivalent PO_4 ions. Cassie and Palmer[80] have shown mathematically that the increased surface activity of the paraffin salts by the addition of electrolytes, can be accounted for by the change in the potential energy of the surface film, brought about by the increased concentration of the ions of opposite charge in the aqueous solution. Aickin and Palmer[81] in 1944 published a theory to explain the differences in the effect of various added ions on the surface activity of the anionic detergents, taking into account the charge and the size of the ions. Divalent and trivalent ions are

better flocculating agents and hence the maxima in the dispersive power curve will be reached earlier i.e., at a lower concentration,



Lamellar Micelle according to McBain.

than for sodium.[82] Similar effects of added electrolytes have been

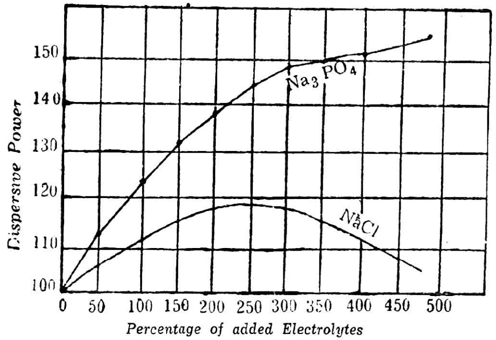
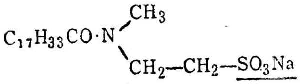


Fig. III. Effect of salts on the dispersing power of Belloid T. D.

observed in the case of cation active and nonionic type of detergents also.[83, 79]

Effect of pH on the Performance of Detergents:—Snell[84] has pointed out that pH of the solution is a rather unimportant factor for maximum detergency. The attempts to control pH concentrations of modified soda systems or soap systems in laundry practice are not successful.[85] Palmer's[86] observation that the detergency of sulphated alcohols decreased rapidly with fall of pH while the increase in pH had but little effect, cannot be of

general application in the light of Scott's work.[44] From Fig IV it is clear that both acids and alkalies (0·2%) exert a salting effect in the case of alkyl chains of short length like C_{10}, C_{11} etc., thereby considerably increasing their wetting properties. When the length of the carbon chain increases, addition of not only the acid but also of alkali depletes the wetting properties. This is in support of the fact that the alkali reduces the polar repulsion of the sulphonate group thereby favouring flocculation. Moreover Palmer has pointed out[86] a similar fall of detergency with increase in pH, in the case of Igepon T used for scouring wool. This phenomenon is explained by the fact that the change towards alkaline pH may cause the polar end of the Igepon molecule



to reverse its orientation from water towards wool surface.

From the above observations it is clear that pH effects on the detergents must be considered in conjunction with salt effect and the hydrophilic—lyophilic balance.

Synergistic Effect of Builders on Detergents:—The general subject of the influence of builders on synthetic detergents has been reviewed by Harris.[87–87a] The demerits of some of the detergents like 'Santomerse' which are affected by hard water, can be improved by building with sodium poly phosphate. By properly building the detergent with appropriate inorganic ion exchangers like Calgon,

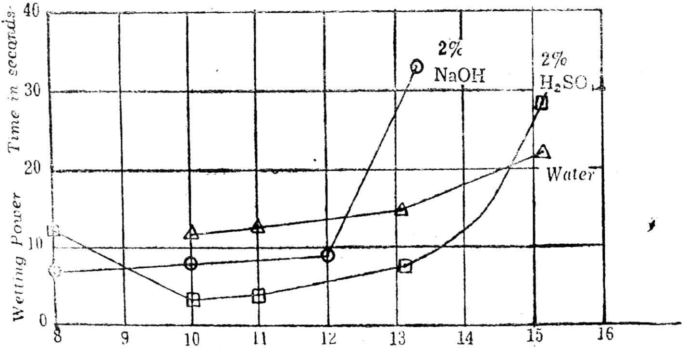


Fig. IV. Influence of alkyl chain length and pH in detergency at 2·5% concentration.

Number of C. atoms in the mole,

it has been possible to achieve, with only 40% active base content, efficiencies higher than for pure detergents alone ;[43] and some of the built detergents can compete with soap under any working conditions.

Besides building up with inorganic salts like sodium sulphate, sodium silicate, and sodium phosphate, building one detergent with another has also been investigated. Barker[88-89] reports that the detergency of anionics like alkyl aryl sulphonates can be improved by 30 — 40% by mixing them with nonionics like Renex in the ratio of 4 : 1. The efficiency of this mixture is shown to improve further by building the same with proper inorganic salts. Lewis Mcdonald[90] has shown that soap containing 3—4% of synthetic detergent gives better performance in hard water than soap or detergent alone.

Industrial Evaluation of Commercial Detergents: —The tendency to evaluate detergents in terms of their performance with fabrics, has gained currency because laundry is such a large consumer of detergents. However, so great is the variety of surface active agents, and the types of surfaces to be cleaned that it is futile to attempt to predict from the data of a launderometer test, the performance of a detergent for a specific case. Thus soaps hailed as good all—purpose detergents, may act as negative detergents, as Snell points out[56] in cleaning metal surfaces. Realizing the futility of devising a universal test, several workers have now turned to laboratory methods which would parallel specific service conditions, under which the particular detergent is to be used. In several instances attempts have been made to correlate the comparative values for specific properties of different detergents with their constitution. Without going into the details of these evaluation methods, it is worth while to mention a few examples :

(a) The utility of detergents in the manufacture of paints and inks can be determined by carrying out simple tests of dispersive power, or protective action on standard suspended soil, say ilmenite or umber[57-59] using an electric turbidimeter or similar appliance for measurement of residual suspended matter.

(b) The efficiency of detergents in operations like prevention of scale formation or the scale removing power of the detergent can be evaluated by measuring the power of the detergent to solubilize or disperse the calcium soap.[60-61]

(c) The performance of various detergents in textile processing can be compared by simple tests like rapidity of wetting or

sinking time, Herbig Number, or drop number measured with Donnan pipette, shrinkage, and centrifugal tests.[62–64]

(d) The effects of detergents in dyeing baths can be investigated by a study of solubilization of water-insoluble dyes in aqueous solution of different detergents under differing conditions like pH, added salts, concentration of detergent, and temperature.[65–67]

(e) The efficiency of the detergents as frothers in flotation processes, can be estimated by noting the amounts of foam produced, under standard conditions. Though the measure of froth is not indicative of the efficiency in laundering,[68] yet good lather will serve as a cushion to prevent excessive wear of the fabric during the washing operation. Beyond the small amount necessary for this purpose, volume of foam has no function, except its advertisement value.[69]

(f) The detergents to be used in laundry can be compared for their efficiency with the help of a launderometer which enables the reproduction of similar conditions as in the laundry practice. The degree of cleansing is determined by measuring with a photometer the reflectance of the test specimen fabric, before and after washing :—

$$\% \text{ of soil removed} = \frac{R_w - R_s}{R_o - R_s} \times 100$$

Where R_w = Reflectance of washed fabric,
R_s = Reflectance of soiled fabric,
R_o = Reflectance before soiling.

Such tests have been elaborately described by Bacon and others.[70, 71, 92]

(g) The Atlas Powder Co.[72] have now published empirical tables of HLb numbers of detergents (indicative of their Hydrophilic—Lyophilic Balance). Different detergents have different HLB numbers, and specific limits of HLB numbers favour particular industrial purposes. A rough choice of a detergent or a proper blend of detergents for any specific purpose can be made by reference to these tables.

(i) A fair idea of all-round general detergency power of any compound can be had,[69] by the determination of such physical properties like adhesion tension of soil-fabric and detergent-fabric, detergent-oil interfacial tension, contact angle between soil/fabric etc.

At any rate, there is so much of activity in the field of evaluation of detergents to find some simple formula, that we may,

with F. D. Snell[73] speculate, that, some day there will be in use a formula for evaluation of detergency at a glance such as $D = X + Y + Z + W$, where $X =$ wetting power, $Y =$ dispersive power, $Z =$ Solubilizing power, $W =$ a function of the contact angle against the base.

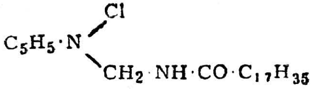
Analysis of Commercial Detergents:—Owing to the large variety of compositions and the possibility of large variety of added builders, no systematic analysis has been devised. A few attempts have been made on qualitative analysis.[74-78] However any scheme of quantitative analysis of commercial synthetic detergents, to be satisfactory, must include estimations of (a) Total degree of sulphonation or sulphation, (b) amount of soap, (c) free fatty acids, (d) neutral oil, (e) water, (f) inorganic builders, (g) unsaponifiable matter, (h) identification of the original oil, or fatty product. Such a scheme has been described by Griffith.[79]

Industrial Uses of Synthetic Detergents:—The limited space of this article does not permit any exhaustive treatise on the uses of detergents. Moreover, so multifarious are the uses of surface active agents, that it is impossible to draw a limiting line. The commercial uses of detergents are listed in the articles of Samuel Leuhr,[91] Paice,[4] Borghetty,[38] Schwartz.[92] The major consumers of these products at present are textile, wool, leather, paints and inks, metal cleaning, and laundry industries.

(a) Detergents find use in textile industry (1) for lubrication of yarn by producing a de-electrifying type of oil/water emulsions, non-ionics being suggested by Speakman[89] as the best for the purpose; and (2) for scouring, synthetic detergents built up with polyphosphates, and sodium carboxy methyl cellulose, proving as efficient if not more than soap in washing off heavy dirt.[5]

(b) Wetting powers of secondary alkyl sulphates are highly satisfactory in the wet chlorination for shrink-proofing of wool, and in the carbonizing bath for uniform wetting of wool with sulphuric acid.

(c) Cationics are recommended for softening the 'handle' of fabric adversely affected by processes like chlorination. But the cationics decrease the fastness of dyes. On cotton better results are achieved by the use of closely related compounds like Stearamidomethyl pyridinium chloride,



which improves the handle without any deleterious effect on the dyes. But it is ineffective in wool.

(d) Cation active detergents are also largely used in coating fabrics with water repellents.

(e) Non-ionic type of detergents are used in dye baths to bring about uniform spreading of dyes at the cost of rapid penetration.

(f) Alkyl aryl sulphonates are used as the contact agents in the Twitchell's process of fat-splitting.[93,94]

(g) Anionic type of detergents find use in the fur and leather industries for soaking, scouring, and dyeing etc.[95, 96]

(h) Largest consumer of detergents is the laundry. During war time and after, there has been vigorous work to make the synthetics suitable for the Navy's sea-water laundering.[97] Lever Bros. have proposed to market briquettes of detergents, resembling soap, with blue flourescent compounds incorporated for imparting extra whiteness for white fabric.[98]

(i) Other industries which make use of detergents for some purpose or other are electroplating, cosmetics, dairy, canning and food industry, horticulture, photography, rubber, plastics, cement and concrete,[99] paper pulping, fire fighting,[100] and radiator cleaners.[101]

(j) Cationics are being widely explored in the pharmaceutical field for use in skin medicaments,[102] cleansing scalp, and for bactericidal purposes.[103–108]

One of the interesting uses of detergents has been pointed out by Saylor[109] of the Parker Pen Co. Besides preventing feathering of ink on the paper, detergents inhibit the clogging of the nibs by caustic inks. Clogging is due to combination of CO_2 with the caustic alkali. The detergent molecules are oriented with the non-polar groups on outside so that they act as a hydrocarbon seal which prevents the penetration of CO_2 into the interior.

(k) Demand for detergents for domestic use is as considerable as that from laundries. But the synthetic detergents which could not be presented in the form of bars or blocks could not easily attract the house-wife who is long accustomed to briquettes of soap. At present there are about only two firms who have placed solid washing compositions in the market[110–112] which are adapted for washing cans, bottles and sinks.

The Present Status and the Future Trends:—The detergent market today is dominated by soap, in spite of the latter's drawbacks, namely the instability in the acid ranges, and precipitation by the polyvalent mineral ions in hard water. Nevertheless, if we project the present-day position of the synthetic detergents against the picture half a dozen years back, we have ample grounds to hope that the synthetics will have a brighter future. To compete successfully with soap in the market, the conditions to be fulfilled by the detergents are: (1) the materials must be presentable in the form of bars or tablets to be popular with the domestic consumers who are long accustomed to solid soap; and (2) they must be more effective or at least as effective as soap in the removal of heavy dirt from cotton; and lastly; (3) their cost must be comparable with that of soap. Property-wise many synthetics can excel soap especially in hard water areas. This has been made possible by the fact that the synthetics can be built up with appropriate inorganic fillers and sodium carboxy methyl cellulose, when they show better detergent properties than the pure detergents. Though initially the detergent-industry banked on the natural fatty oil resources, and the cost of detergents had ranged much higher than that of soap, the discovery that petroleum hydrocarbons and coal can be sources of raw materials for this industry, has enabled a good many of the detergents to compete with soap in cost. Hersberger and Neidig[10] have surveyed the present status of the synthetic detergent industry in America. Table 1 gives a summation of the synthetics production on the basis of 100% active base. The figures are expressed in pounds.

Table 1. Annual production of synthetic detergents in U.S.A.

	1946	1947	1948
Alkyl aryl sulphonates ..	52549,000	62365,000	82000,000
Sulphated alcohols ..	45000,000	62000,000	65000,000
Miscellaneous sulphonates ..	71118,000	85793,000	85000,000
Nonionics ..	14955,000	16924,000	21000,000
Total ..	183622,000	227082,000	253000,000

A comparison of 3 years of soap sales and synthetic market of U.S.A., which is now the biggest producer of synthetic detergents is shown in Table 11.

Table 11

	* Soap in lbs.	Synthetics on 100% base basis (expressed in lbs.)
1946	257,0000,000	183,622,000
1947	310,0000,000	227,082,000
1948	276,0000,000	253,000,000

* These figures [113] include alkalies, dyes, fillers, and other items which are incorporated in soap.

In 1948 the total sale of synthetic detergents on the basis of 100% base was about 10% whereas in 1946 it was only 7% of the total soap sale. It will also be interesting to note that the soap sales of 1948 are about 11% below that in 1947. This is a clear trend of the detergents gaining ground and soap sales falling down. It is now expected that in 1950 the synthetics will be 40% of the soap market.

The World-consumption of detergents will certainly be on the ascent so long as the present shortage of oils and fats which are the raw materials of soap industry, continues. It is predicted that this shortage may continue till 1952, or even upto 1960. In India, which is one of the biggest growers of oil seeds of the world, changes have occurred, both due to increase of population and increase in per capita consumption, which have necessitated a cut down in the exports. In 1937 the production was 1809,000 tons of oil and the export was 470,000 tons, whereas in 1947 the production was 1700,000 tons and the export only 80,000 tons. (Foods and Agricultural Organization's figures for oils and fats in India). So in a decade there was a loss of 400,000 tons of oil to the soap factories of the World. To replace this loss United Kingdom tried to implement a 5-year plan for growing ground nuts in S. Africa, a project which was expected to yield 600,000 tons of nuts corresponding to 250,000 tons of oil. But the latest information shows that it is not a success. Hence the soap industry will be experiencing the shortage of raw materials for some more time and this is bound to give a fillip to the synthetic detergent industry.

With the present 'Grow-More Food' campaign of the India Government, pre-war surplus of oil and much more will be available to the Indian oil industry.

It is also apparent that our large natural resources of non-edible oils (mainly forest products) like mowrah punnal, niger neem, Dhupa fat etc., can be diverted to the manufacture of several synthetic products of immense use and great demand in industries

like textiles, leather paint etc. The unhealthy dependence of our vast textile, leather, and paint industries on the foreign market for the very important detergents, textile auxiliaries, and emulsifiers, must be remedied as early as possible. In a self-Sufficiency programme, it must be one of the leading items to work out methods for processing our vast and perennial resources of non-edible oils to yield synthetics like detergents, plastics, paint vehicles, etc.

I wish to acknowledge my grateful thanks to Dr. M. A. Govinda Rao, the Director, Alagappa College of Technology, University of Madras, for lending me many technical pamphlets and for his guiding criticisms in the preparation of this review.

References

1. Rideal, E. K. Chem. & Ind. *26,* 1948, pp. 403.
2. McBain, J. W. 'Advances in Colloid Science', Inter Sc. Publ., pp. 99-139, ,Vol. 1).
3. Boland, C. E. Can. Chem. Process Ind., 1948, *32,* 1135-8.
4. Braybrook, Chem. & Ind., 1948, *26,* 404.
5. Sarin and Uppal, Ind. Eng. Chem., 1941, *33,* 666-8.
6. Eric Hardy, 'Perfumery and Essential Oil Record', 1942, *33,* 203. (C. A. 1942, *36,* 6267).
7. Kind, W. Fette u. Sefen, 1942, *49* 708-11, (C.A. 1943, *37,* 6371).
8. Patel, C. V. Indian Text J. 1938, *49,* 27-9, (C.A. 1939, *33,* 776).
9. Linsenmeyer, Melliand Textilber, 1940, *21,* 468-69.
10. Hersberger, and Neidig, Chem. Eng. News, 1949, *27,* 1646-50.
11. Snell, F. D. J. Am. Oil Chem. Soc., 1949, *26,* 338.
12. Snell, F. D. Ind. Eng. Chem., 1943, *35,* 107-117.
13. Paice, E. S. Chem. and Ind., 1948, *26,* 691-4.
14. Naik, K. G. and Desai, C. M. J. Sci. Ind. Res., 1948, *7,* 193-7.
15. Padgett, Ind. Eng. Chem., 1940, *32,* 204-8.
16. Dreger, Keim, Miles, Shedloosky, and Ross, Ind. Eng. Chem., 1944, *36,* 610.
17. "TERGITOLS". A technical pamphlet of the 'Cyanamide Corp".
18. Winsor, P.A. Nature 1946, *157,* 660. (C. A. 1946, *40,* 5320).
19. Snell, F. D. Soap and Sanit Chem. 1943, *19,* No. 17, pp. 31-33.
20. Shirolkar and Venkataraman, K. J. Soc. Dyers and Col., 1941, *57,* 41.
21. Uppal and Venkataraman, J. Soc. Dyers and Col., 1939, *55,* 125.
22. Rao, S. N. J. Ind. Text, 1949, *59,* 346.
23. Eckey, E. W. Ind. Eng. Chem. 1948, *40,* 1183.
24. Eckey and Formo, J. Am. Oil Chem. Soc., 1949, *26,* 207.
25. Adolf Grun, U.S.P. 2138917, Dec. 6th, 1938, to American HYALSOL Corp.
26. Can. P. 426102 March 13th, 1945, Colgate, R. B; Dreger, and Russel, K. L. to Colgate Palmolive Peet Co. (C. A. 1945, *39,* 2664).
27. Can, P. 425822 Feb. 27, 1945. Muncie, F. W. to Colgate Palmolive Peet Co.
28. Victor Boyer, Rev. Pro. Chim. 1944, *47,* 1-2, 17-19, (C.A. 1947, *41,* 7778).

29. Matetsku and Raikhlin, Tekstil Prom. 1941, 5, 35-6. (C.A. 1944, *38*, 3152).

30. Speakman, Chem. and Ind. 1948, *26*, 456.

31. Mehta and Trivedi, J. Soc. Dyers and Col., 1940, 56, 343.

32. Warwicke, Soap 1939, *15*, No. 10, 25-6.

33. Borghetty, J. Am. Oil Chem. Soc., 1949, *26*, 319.

34. "ARQUADS" Technical pamphlet, Armour Chem. Division, Armour & Co., publishehd in 1948.

35. Jean Ripert, Soap and Perfumery Cosmetics, 1946, 19, 552-5. (C.A. 1946, *40*, 7667).

36. Jack Dollinger, Soap Sanit Chem., 1949, *25*, No. 1, 37-9. (C.A. 1949, *43*, 2002).

37. McBain and Marsden Jr., J. Chem. Phys., 1947, *15*, 211-212.

38. Gonick and McBain, J. Am. Chem. Soc., 1947, *69*, 334-6.

39. Young and Coons, "Surface Active Agents", Chemical Publ. Co., N. Y., 1945 Edition, pp. 108-110.

40. Griesinger, W. K., Nevinson, J. A. and Gallagher, G. A. J. Am. Oil Chem. Soc., 1949, *26*, 241-4.

41. Snell, F. D. Chem. Eng. News, 1949, 27, 2257.

42. Scott, W. D. & Roebuck, D. S. P., Chem. & Ind. 1948, *26*, 627-31.

43. Preston, J. Phys. Colloid Chem., 1948, *52*, 84.

44. Gruntfest and Young, J. Am. Oil Chem. Soc., 1949, *26*, 236-8.

45. Lomas, H. J. Soc. Chem. Ind., 1949, *68*, 37-40.

46. Wetting and Detergency Symposium, Brit. Sect. Interntl. Soc. of Leather Trades Chem., 1937, Feb. 19th, pp. 153-62, (C.A. 1940, *34*, 2098).

47. G. S. Hartley, J. Chem. Soc. 1938, pp. 1968.

48. Kiessig and Philippoff, Naturwissenschaften, 1939, *27*, 593.

49. Ralston, A. W. Ann. N. Y. Acad. Sci. 1946, *46*, 351-69 (C.A. 1946, *40*, 4583.

50. Bernal, Trans. Farad Soc., 1946, *42*, 197.

51. Harkins, Mattoon and Corrin, J. Coll Sci., 1946, *1*, 105.

52. Vetter, J. Phys. Colloid Chem., 1947, *51*, 262.

53. Hartley, G. S. NATURE 1949, *163*, No. 4150, p. 767.

54. Klevens, H. B. J. Am. Oil Chem. Soc., 1949, *26*, 456.

55. McBain and McBain, J. Am. Chem. Soc., 1936, *58*, 2610.

56. Reich, Irving and Snell, Ind. Eng. Chem., 1948, *40*, 1233-7.

57. Fischer and Jerome, Ind. Eng. Chem., 1943, *35*, 336-343.

58. Powny and Noad, J. Text Inst., 1939, *30*, 157-71 (C.A. 1940, *34*, 2098).

59. Snell, Irving, Reich, J. Soc. Chem. Ind., 1949, *68*, 98.

60. Sisley, Corps. Gras., Savons, 1944, *2*, 77-9. (C.A. 1946, *40*, 5271).

61. Ramachandran, S. R., Uppal, and Venkataraman, J. Soc. Dyers & Col., 1938, *54*, 520.

62. Mehta, T. N., and Trivedi, D. M. Melliand Textilber, 1940, *21*, 117-19, (C.A. 1940, *34*, 6087).

63. Forster, Uppal and Venkataraman, J. Soc. Dyers & Col. 1938, *54*, 465.

64. Sisley, J. P., Corps. Gras., Savons, 2, 1944, No. 1, 13-19. (C.A. 1946, *40*, 5271).

65. McBain, J. W., Merril, Jr., Ind. Eng. Chem., 1942, *34*, 915-19.

66. McBain and Richards, Ind. Eng. Chem., 1946, 38, 642-6.

67. Lambert & Busse, J. Am. Oil Chem. Soc., 1949, *26*, 289.

68. Edmund Walter, Fette u. Seifen, 1941, *48*, 622-24.

69. Kenneth Tomlinson, Manufg. Chem., 1949, Oct., Vol. 20, No. 10, pp. 477.

70. Vaughn, Vittone & Bacon, Ind. Eng. Chem., 1941, *33*, 1011.

71. Bacon, O. C.; Smith, J. E. Ind. Eng. Chem., 1948, *40*, 2361-70.

72. "Atlas Surface Active Agents" Technical pamphlet (second printing), pp. 13-22—(Atlas Powder Co., Delaware).

73. Snell, F. D. Manufg. Chem., 1949, *20*, No. 8, 389.

74. Wurzschmidt, Chimie Analytique, 1947, p. 44 (Oil and Colour Trades. Apr. 1947, p. 675.)

75. Vander Hoeve, J. A., Rec. Trav. Chim., 1948, *67*, 649-64.

76. Hoyt L. F., J. Am. Oil Chem. Soc., 1947, *24*, 54-6.

77. Nandet, M., & Desnaelle, P., Oleagineaux 1948, *3*, 458-63. (C.A. 1949, 43, 3217).

78. Shirolkar and Venkataraman, J. Ind. Chem. Soc., Ind. Edn., 1941, *4*, 61.

79. Griffith, Manufg. Chem., 1949. *XX*, *8*, 365.

80. Cassie & Palmer, Trans. Farad. Soc., 1941, 37, 156.

81. Aickin & Palmer, Trans. Farad. Soc., 1944, 40, 116. (C.A. 1944, *38*, 4854).

82. Palmer R. C., J. Soc. Chem. Ind., 1941, *60*, 56-60.

83. Hill & Hunter, Nature, 1946, *158*, 585.

84. Snell, F. D., Ind. Eng. Chem., 1932, *24*, 76-80, 1051-57.

85. Bacon, L. R., Hensley, J. W., Vaughn, T. H., Ind. Eng. Chem. 1941, *33*, 723.

87. Harris, J. C., Oil and Soap, 1946, *33*, No. 4, 101.

87a. Harris, J. C., Soap, *19*, 1943, No. 8, 21-24, 29-31, (C. A. 1943, *37*, 6763).

88. Barker, J. Am Oil Chem. Soc., 1949, 304.

89. Barker, G. E. Proc. of the Scientific Sect. of the Toilet Goods Association, 1948, No. 9-May.

90. Louis MacDonald, Soap Sanit. Chemicals, 1945, *21*, No. 12, 41-3, (C. A. 1946, *40*, 748.)

91. Samuel Leuhr, Chem. and Ind., 1941, *19*, 497-500.

92. Schwartz, A. M., J. Am. Oil Chem. Soc., 1949, *26*, 212-215.

93. Hilditch. "Industrial Fats and Waxes", 1943, Edn. pp. 275. (Bailliere, Tindal & Cox).

94. Bailey, "Oil and Fat Products". 1945, Edn. pp. 668-669. (Inter. Science Publ. N.Y.).

95. Smith, Soap Sanit. Chem., 1949, *25*, No. 5, 33-4.

96. Smith, P. I., Soap Sanit. Chem., 1946, *22*, No. 5, 46-47. (C. A. 1946, *40*, 4237.)

97. Vaughn, T. H., Hill, S. E., Smith, C. E., McCoy, L. R. Ind. Eng. Chem., 1949, *41*, 112-19.

98. Richard Thomas, (to Lever Bros., Unilever, Ltd.), Br. P. 584424, Jan. 1947. (C. A. 1947, *41*, 3644.)

99. Wetton, R. G. (to Keasbey and Mattison Co.), U. S. P. 2315029. (C. A. 1943, *37*, 5568.)

100. Parker, W. W. J. Chem. Educn., 1947, *24*, 615-17.

101. Milton, A. Lesser, Soap Sanit. Chem. 1949, *25*, No. 5, 40-3. (C. A., 1949, *43*, 5214.)

102. Quimby, S. and Fiero, G. W., N. Y. State, J. Med., 1943, *43*, 157-9. (C. A., 1943, *37*, 1561.)

103. Miller, B. F., and Baker, Z., Science, 1940, *91*, 624-5. (C. A .1940, *34*, 5874.)

104. Gonzalez and Madeiros, Rev. Med. Y. Alimentacion (Santiage, Chile), 1942-43, *5*, 232-5. (C. A. 1945, *39*, 325.)

105. Kruegar, A. P., U. S. Navy Med. Bull., 1942, *40*, 622-31. (C.A. 1942, *36*, 6747).

106. Stephen, R. M., and Miller, B. F., Proc. Soc. Exptl. Biol. Med., 1944, 55, pp. 101-104. (C. A. 1944, *38*, 2124.)

107. Klein, M., Kalter, S. S., and Stuart Mudd, J. Immunol, 1945, *51*, 389-96. (C. A. 1946, *40*, 2195.)

108. Klein, M., and Stevens, D. A., J. Immunol, 1945, *50*, 265-73. (C. A. 1945, *39*, 3362.)

109. Reynolds, W., Ind. Eng. Chem. News Ed., 1946, Apr. pp. 916.

110. U. S. P. 2382163, Aug. 1945, MacMohan, J. D., to Mathieson Alkali Works. (C. A. 1945, *31*, 5521.)

111. John Brodman, and Fred. Forrest, to lever Bros., U. S. P. 2400871, May 8th, 1946. (C. A. 1946, *40*, 5274).

112. I. G. Farbenind, A. G., Belg., 446970, Sept. 1942. (C. A. 1945, *39*, 1070.)

113. Association of American Soap and Glycerine Producers.

93187

Printed and Published by G. Srinivasachary, B.A., at G. S. Press,
21, Narasingapuram Street, Mount Road, Madras.

PUBLICATIONS

OF

THE INTER-UNIVERSITY BOARD, INDIA

		Price Rs.	A.	P.	
1.	**Handbook of Indian Universities** ..	3	0	0	*or* 5s
2.	**Facilities for Oriental Studies and Research at Indian Universities** ..	0	8	0	
3.	**Facilities for Scientific Research at Indian Universities** ..	0	12	0	
4.	**Bulletin of the Inter-University Board, India,** Nos. 1 to 13 ..	1	0	0	*each*
5.	**Biological Outlook on Life and its Problems.** By J. ARTHUR THOMSON, M.A., LL.D., Regius Professor of Natural History, University of Aberdeen ..	0	2	0	
6.	**Second, Third and Fourth Conference of Indian Universities** ..	1	0	0	*each*
7.	**Training of Teachers in Indian Universities** ..	0	8	0	
8.	**Bibliography of Doctorate Theses in Science and Arts (Accepted by Indian Universities from January 1930 and from 1934)** ..	0	8	0	*each*
9.	**Annual Report of the Inter-University Board for 1940-41** ..	1	0	0	

[POSTAGE AND V. P. CHARGES EXTRA]

Available from :

BANGALORE PRESS,
"LAKE VIEW", MYSORE ROAD,
BANGALORE CITY.

JOURNAL OF THE MADRAS UNIVERSITY

ISSUED TWICE A YEAR

Subscription Rs. 4 per annum.

(Rs. 3 for Principals and Teachers of Colleges)

Back Numbers contain Important Contributions from well-known scholars on Literature, Philosophy, Mathematics, Botany, Zoology, History, Economics, Politics and Ethnology.

Among the Supplements are "The Origin of Saivism", "Vyasa Siksha" (Sanskrit), "History of Shakespearean Criticism", "Samkhyakarika", "Vayupurana", "Sahitya Ratnakara" (Sanskrit), "Indian Federation", and "Handloom Weaving in the Madras Presidency".

BACK NUMBERS ARE AVAILABLE

Apply to the Editor :

JOURNAL OF THE MADRAS UNIVERSITY,

University Buildings,

MADRAS.